THE NEUROPHYSIOLOGY AND BEHAVIORAL PHARMACOLOGY OF MEMORY ENHANCEMENT AND MEMORY DEFICITS IN THE DENTATE GYRUS

by

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ABSTRACT

Memory deficits are a devastating consequence of many neurological disorders including Alzheimer's disease (AD) and epilepsy. Currently approved treatments for memory impairment in AD are few in number, mechanistically homogenous, and only marginally effective, and there are no approved treatments for memory deficits in epilepsy. Thus, new treatments are needed. One path to new therapy development is to first obtain a mechanistic understanding of existing treatments, including those in development, in order to "fine-tune" their targeted effects or discover new therapeutic approaches. Another strategy is to investigate the underlying pathology causing memory dysfunction in order to identify new therapeutic targets. Research in this dissertation utilized both strategies. Chapter 2 used *in vitro* electrophysiology and immunohistochemistry to investigate the neurophysiology of 5-HT_6 receptors, a target with widely reported nootropic effects. Results from these studies suggest that 5-HT_6 receptors bidirectionally modulate inhibitory synaptic transmission in the dentate gyrus (DG) via their direct effects on excitatory mossy cells thought to drive inhibition in the DG. Chapter 3 tested the procognitive and anticonvulsant effects of 5-HT_6 receptor antagonists in J20 mice, a transgenic model with pathological hyperexcitability thought to contribute to disease progression. Systemic treatment with a 5-HT_6 receptor antagonist improved spatial pattern processing in nontransgenic (NTG) mice, but not in J20 mice. Additionally, J20 mice had significantly a lower seizure threshold during the minimal

clonic seizure test compared to nontransgenic mice. However, 5-HT_6 receptor blockade had no affect on seizure threshold in either AD genotype, but did exhibit strain and ligand-dependent proconvulsant effects in naïve and otherwise healthy mice. Lastly, studies in Chapter 4 found that corneal kindled mice, which are a model of seizure development, exhibited DG-associated spatial pattern processing impairments. *In vitro* electrophysiology in acute hippocampal brain slices revealed DG granule cells in corneal kindled mice were hyperexcitable and had long-term potentiation deficits associated with DG-mediated cognitive dysfunction. Together, these results suggest that targeting disinhibition and aberrant hyperexcitability may be viable therapeutic targets for treating memory dysfunction in epilepsy. Ideally, new treatments for memory impairment should be both procognitive and anticonvulsant.

This dissertation is dedicated to my Mom and Dad.

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CHAPTER 1

INTRODUCTION

Memory deficits are a devastating consequence of many neurological disorders, affecting patients with dementia, epilepsy, Down's syndrome, autism-spectrum disorder, Parkinson's disease, as well as patients with psychiatric disorders (Selkoe, 2001; Helmstaedter, 2002; Cirillo and Seidman, 2003; Vicari et al., 2005; Boucher et al., 2012; Skodzik et al., 2013; Pauls et al., 2014). Not only does memory impairment interfere with normal functioning, but as memory deficits worsen they become dangerous, and are thus among the primary causes for admission into residential nursing homes (Andel et al., 2007). It was estimated that delaying admission into a residential care facility for dementia patients in the U.S. by only 1 month would cut healthcare spending by $4 billion (Gold and Budson, 2008). Thus, treatments that attenuate memory deficits will improve quality of life and diminish the heavy financial and emotional burden of neurological disease. New treatments are needed because currently available therapies for memory deficits are few in number, mechanistically homogenous, only marginally effective, and present with side effects (Raina et al., 2008; Nygaard, 2013). Towards this end, the scope of this dissertation is to better understand the neurophysiology and behavioral pharmacology of memory enhancement and memory impairment in order to highlight new therapeutic targets and perhaps guide the clinical use of current treatments.

Memory Deficits in Disease

Memory deficits in AD typically begin with mild cognitive impairment (MCI), first affecting temporal lobe-dependent episodic memories such as misplacing keys, forgetting an appointment, or difficulty remembering a particular conversation – events that are typically associated with normal aging (Frisoni et al., 2010). However, as AD progresses, worsening episodic memory is accompanied by semantic memory impairments such as difficulty with verbal recall and object recognition. At this point, patients are usually prompted by loved ones to seek medical attention, resulting in a diagnosis of MCI. Identification of the constellation of symptoms required for a diagnosis of MCI and probable AD can be performed during a routine visit with a clinician (a definitive diagnosis of AD requires postmortem examination). Cognitive tests such as the Mini Mental Status Exam (MMSE) or the Alzheimer's Disease Assessment Scale with cognitive measures (ADAS-cog) are sensitive to subtle changes in declarative memory function and can be used to make an early and reliable diagnosis. MCI progresses to probable AD when more global cognitive deterioration occurs, indicated by deficits in attention, visuospatial memory, and executive function that interfere with one's ability to function independently (McKhann et al., 2011).

AD is not the only neurological disease accompanied by memory impairment. A survey of over 1000 epilepsy patients found that cognitive difficulty was the greatest concern for patients with epilepsy, and "the ability to remember things" was ranked highest amongst qualities affected by epilepsy (Fisher et al., 2000). Another survey reported that over half of all epilepsy patients regarded memory impairments as a moderate-to-severe "nuisance" (Thompson and Corcoran, 1992). Moreover, children with

temporal lobe epilepsy (TLE) also suffer from memory disturbances that contribute to poor performance in school and the lifelong socioeconomic difficulty patients with epilepsy face (Rzezak et al., 2014). Given the significant impact memory impairment in AD, epilepsy, and other neurological disease has on quality of life, and the socioeconomic ramifications endured by patients, their families, and society, new treatments are urgently needed. Researchers tasked with new therapy development face an onerous challenge and can adopt a variety of strategies to discover new treatments for memory impairments in disease.

Top-Down and Bottom-Up Approaches to New Therapy Discovery for Memory Deficits in Disease

Perhaps the most common approach to new therapy development is to elucidate the underlying pathophysiological mechanisms of disease to yield effective targeted therapies. This strategy, which can be thought of as a bottom-up approach, has and will likely continue to yield beneficial therapies that dampen, prevent, or reverse disease symptoms, however, the complex heterogeneity of neurological disorders makes this approach a formidable endeavor that will likely take years, if not decades before new treatments are available. Nevertheless, a mechanistic understanding that targets the underlying pathophysiology is a cornerstone of disease research and may be necessary for prevention or complete remediation of symptoms.

An alternative approach is to study existing treatments, including those in development, that have a more general impact on memory, and reverse-engineer their therapeutic effects. The rationale behind this strategy, which can be thought of as a top-

down approach, is that a mechanistic understanding of treatments already shown to improve memory may provide a roadmap for their beneficial effects, and thus, lead to new therapies that "fine-tune" existing treatments and highlight novel therapeutic targets.

As it relates to the research in this dissertation, both top-down and bottom-up strategies were employed. The top-down approach in this study dissects the physiology and function of 5-HT_6 receptors, a serotonin (5-HT) receptor subtype known for its memory enhancing effects. 5-HT_6 receptor cell-type specific receptor expression patterns and their effects on synaptic transmission in brain areas critical for memory formation are poorly understood. Thus, the first goal of this study was to provide an anatomical and physiological blueprint for the memory enhancing effects of 5-HT_6 receptor antagonists in the dentate gyrus (DG), a subregion of the hippocampus that is critical to new memory formation. The second goal was to examine the role of 5-HT_6 receptors on spatial pattern processing and seizures in a transgenic model of AD. Results from this investigation are expected to direct the discovery of new therapeutic approaches for memory impairment in neurological disorders such as AD and epilepsy, and inform the current clinical use of 5-HT_6 receptor antagonists.

The bottom-up approach in this study investigated brain region-specific pathophysiology associated with spatial pattern processing deficits in a corneal kindled mouse model of network hyperexcitability (Rowley and White, 2010). Although epilepsy patients and animal models of epilepsy exhibit memory deficits associated with neuron loss and hippocampal sclerosis, some epilepsy patients exhibit memory impairments in the absence of overt brain damage (Berg et al., 2009; Berg, 2011). Although it is ideal to treat all patients with memory deficits, it may be a more manageable endeavor to first

develop treatments for memory impairment in animal models with less neuron loss. Thus, we wanted to test whether corneal kindled mice, which are a readily implemented model of network hyperexcitability shown to lack overt hippocampal damage (Loewen et al., 2016), exhibit cognitive dysfunction in the absence of extensive neuron loss. Further, we investigated the underlying pathophysiology that may contribute to these deficits. Results from this investigation are expected to inform the pathophysiology underlying memory deficits and potentially provide a model to test therapies that may reverse seizure-related memory impairment.

An Overview of the Serotonergic System and the 5-HT_6 Receptor Subtype

The Serotonin System

Understanding the nootropic effects of 5-HT_6 receptors is a difficult undertaking underscored by the complexity of the serotonin system. Serotonin, 5-hydroxytryptamine (5-HT), is a monoamine neurotransmitter converted from its precursor L-tryptophan by tryptophan hydroxylase (Millan et al., 2008; McCorvy and Roth, 2015). 5-HT is produced and released by neurons originating in brainstem raphe nuclei that send extensive long-range projections innervating almost all major brain regions (Berger et al., 2009). Seven families of serotonin receptors comprised of 14 known receptor subtypes transduce serotonin's powerful effects. Except for the 5-HT_3 receptor, which is a ligand-gated cation-permeable ion channel, all 5-HT receptors are 7 transmembrane spanning domain G-protein coupled receptors (GPRCs) (Millan et al., 2008). 5-HT receptors are primarily found in the frontal cortex, hippocampus, amygdala, striatum, and dorsal horn

of the spinal chord. Their activity influences numerous signaling cascades that affect neuronal excitability, and thus, exert powerful effects of mood, cognition, sleep, pain, appetite, and motor control (Millan et al., 2008). Drugs affecting the serotonin system have remarkable effects on brain function, perhaps best exemplified by the use of selective serotonin reuptake inhibitory (SSRIs) for the treatment of anxiety and depression (Pollack, 2005).

Understanding the effects of serotonin or SSRIs is obscured by the myriad effects of its 14 receptor subtypes, although investigation into their canonical signal transduction pathways has revealed some order (Millan et al., 2008). For instance, 5-HT_4, 5-HT_6, and 5-HT_7 are positively coupled to G_s, such that activation of these receptor subtypes increase intracellular levels of the second messenger cyclic adenosine monophosphate (cAMP). In contrast, 5-HT_1 and 5-HT_5 receptors are negatively coupled to adenylyl cyclase via $G_i/_o$, thus their activation inhibits production of cAMP. The 5-HT_2 receptor is coupled to $G_q/_{11}$, which activates phospholipase C leading to the production of inositol-1,4,5-trisphosphate (IP_3) and the release of Ca^{2+} from intracellular stores. In addition to their prototypical signaling pathways, each receptor subtype has been linked to a diversity of alternate signaling mechanisms. See (Millan et al., 2008) for an extensive review of their downstream signaling cascades.

Attempts to untangle the complexity of the serotonergic system have been greatly advanced by the development of receptor subtype-selective ligands, illustrating the effects of each individual receptor type on brain function. For instance, agonists and / or antagonists of 5-HT_{1A}, 5-HT_4, and 5-HT_6 receptors have cognition enhancing effects in rodents during a variety of learning and memory tasks (King et al., 2008). Additionally,

5-HT_{2A} receptors are thought to play a critical role in executive function (Aznar and Hervig Mel, 2016), whereas 5-HT_7 receptor antagonists are perhaps best known for their role as potential fast-acting antidepressants (Mnie-Filali et al., 2007; Mnie-Filali et al., 2011). Moreover, ligands effecting 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, 5-HT_3, and 5-HT_6 receptors have established anticonvulsant and / or proconvulsant effects, which is not surprising, since serotonin itself is an endogenous anticonvulsant (Hirst et al., 2006; Bagdy et al., 2007; Panczyk et al., 2015). Although the effects of selective serotonin receptor ligands are intriguing and hold great promise for the treatment of neurological disease, the majority of serotonin's actions do not occur in the brain. In fact, > 90% of total body serotonin is found in the gastrointestinal (GI) tract (Mawe and Hoffman, 2013). Although 5-HT receptor ligands have been successfully targeted for the treatment of GI disorders (Mosinska et al., 2016), their expression outside the central nervous system (CNS) hampers their use for treating cognitive dysfunction. Interestingly, 5-HT_6 receptors, known for their beneficial effects on anxiety, depression, obesity, seizures, and perhaps most notably, learning and memory are thought to be expressed exclusively in the CNS, making them an ideal target for treating cognitive dysfunction (although faint 5-HT_6 mRNA expression has been detected in peripheral blood mononuclear cells and in the stomach (Yang and Xiao, 2015)). As a continuation of over two decades of literature attempting to reverse-engineer 5-HT_6 receptor physiology and function, the majority of the work in this dissertation will focus on gaining a better understanding of the neurophysiology and behavioral pharmacology of 5-HT_6 receptors.

An Overview of 5-HT_6 Receptors

The rat 5-HT_6 receptor was first identified and sequenced in 1993 by two independent groups (Monsma et al., 1993; Ruat et al., 1993), followed by the human and murine forms, which were cloned in 1996 and 2001, respectively (Kohen et al., 1996; Kohen et al., 2001). The human 5-HT_6 receptor maps to chromosome region 1p35-p36 and has 89% and 84% sequence homology with the rat and mouse equivalents, respectively. Additionally, the human and mouse form contain 440 amino acids, whereas the 5-HT_6 receptor in rat contains 438 amino acids. In contrast to other serotonin receptor subtypes, the 5-HT_6 receptor has no known isoforms.

Despite being canonically linked to adenylyl cyclase, several G_s-independent signaling pathways are affected by 5-HT_6 receptors. For instance, activation of 5-HT_6 receptors has been shown to increase activity of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway in a Fyn tyrosine kinase-dependent manner (Yun et al., 2007). Although its role in mediating the effects of 5-HT_6 receptor antagonists on cognitive function remains unclear, Fyn has been shown to play an important role in regulating long-term synaptic plasticity and spatial memory (Grant et al., 1992). Additionally, 5-HT_6 receptor activation was recently shown to physically recruit and directly activate mammalian target of rapamycin (mTOR), a pathway involved in cell proliferation, synaptogenesis, neurite outgrowth, as well as neural excitability via ion channel expression (Raab-Graham et al., 2006; Swiech et al., 2008; Meffre et al., 2012). Moreover, treatment with a 5-HT_6 receptor antagonist attenuated aberrantly increased mTOR expression and reversed cognitive deficits in animal models of schizophrenia (Meffre et al., 2012). Lastly, 5-HT_6 receptor antagonists have even been shown to

attenuate serotonin-induced depolarization of neurons expressing 5-HT_6 receptor mRNA in a potassium-dependent manner (Bonsi et al., 2007). Thus, 5-HT_6 receptors affect neurophysiology and function through a variety of complex effects.

Few studies have investigated the role of 5-HT_6 receptors in development. However, their expression in rat begins on embryonic day 12, remains stable throughout adulthood, and is implicated in developmental processes such as interneuron migration, neuronal differentiation, and neurite outgrowth (Riccio et al., 2009; Duhr et al., 2014). Gross anatomical localization of 5-HT_6 receptors using radiolabeled ligands or *in-situ* hybridization suggests high levels of expression in the striatum, caudate-putamen, nucleus accumbens, and olfactory tubercles, with intermediate levels of expression in thalamus, cerebral cortex, and hippocampus in rats and humans (Gerard et al., 1996; Gerard et al., 1997; Roberts et al., 2002; Hirst et al., 2003). Mice exhibit a similar expression profile, with reduced levels of 5-HT_6 receptor expression in the striatum (Hirst et al., 2003). Together, these studies suggest that 5-HT_6 receptors are ideally anatomically localized to modulate cognitive function.

Interest in 5-HT_6 receptor effects on cognition was triggered by attempts to reverse-engineer antipsychotics and antidepressant drugs. Ligand binding studies revealed that tricyclic antipsychotics and antidepressants clozapine, amoxipine, and amitriptyline have a high affinity for 5-HT_6 receptors (Monsma et al., 1993; Roth et al., 1994). These top-down driven studies were the impetus for the discovery and development of a number of 5-HT_6 receptor-specific ligands that have been tested for their behavioral effects in rodents over the last 2 decades. Although a plethora of 5-HT_6 receptor agonists and antagonists have become commercially available, many more 5-

HT_6 receptor antagonists have been developed, likely as a result of many more studies reporting their beneficial effects on cognitive function. Benhamú et al. (2014) and others have written extensive reviews covering the armamentarium of 5-HT_6 receptor ligands and their pharmacology (Benhamu et al., 2014). However, the 5-HT_6 receptor antagonists used in this study, SB-399885 and SB-271046, are perhaps the most widely studied because they exhibit over 200-fold and 50-fold selectivity, respectively, for the 5-HT_6 receptor over other serotonin receptors (Routledge et al., 2000; Hirst et al., 2006). A number of 5-HT_6 receptor agonists have been developed as well, revealing similar effects as the antagonists on certain behavioral assays, particularly those assessing anxiety and depression (Wesolowska, 2010). To date, the most widely studied 5-HT_6 receptor agonists are perhaps WAY-208466, which is used for *in vitro* studies in Chapter 2, and WAY-181187. Both agonists display 50-fold selectivity for 5-HT_6 receptors over other serotonin and monoaminergic receptors, and similar efficacy to the endogenous ligand 5-HT (Schechter et al., 2008). As a result of their development and optimization, 5-HT_6 receptor-selective agonists and antagonists have revealed a wealth of therapeutic potential. Most notably, 5-HT_6 receptor antagonists exhibit beneficial effects in rodent models of anxiety, depression, obesity, and seizures (Fone, 2008; Codony et al., 2011; Meneses et al., 2011); however, they are perhaps best known for their effects on learning and memory.

The Nootropic Effects of 5-HT_6 Receptor Antagonists

5-HT_6 receptor antagonists fall under the moniker of "smart drugs" based on their remarkable cognition-enhancing effects. One the first reports of their nootropic effects

directed antisense oligonucleotides at 5-HT_6 receptors in rats, and tested one of the first centrally acting 5-HT_6 receptor antagonists Ro-046790, and found improved retention of platform location during the Morris water maze (MWM) (Woolley et al., 2001). As 5-HT_6 receptor-selective antagonists became available, studies revealed that acute, subchronic, and chronic administration in naïve, aged, and scopolamine-treated rats exhibited improved acquisition and retention in the MWM (Rogers and Hagan, 2001; Stean et al., 2002; Foley et al., 2004; Hirst et al., 2006). Their nootropic effects are not limited to spatial memory: 5-HT_6 receptor antagonists have been shown to improve performance during associative learning and fear conditioning during passive avoidance and operant conditioning tasks (Foley et al., 2004; Schreiber et al., 2007; Meneses et al., 2011), and reverse natural forgetting and scopolamine-induced deficits during novel-object recognition and social recognition tasks (King et al., 2004; Hirst et al., 2006; Mitchell et al., 2006). Additionally, unpublished data from a collaboration at the University of Utah between Dr. Peter J. West, Dr. Julie R. Korenberg, Dr. Raymond P. Kesner, and Dr. Karen S. Wilcox found that acute systemic treatment with a 5-HT_6 receptor antagonist reversed memory deficits during the novel object place-recognition task in a mouse model of Down syndrome (Korenberg). On the strength of this preclinical data, 5-HT_6 receptor antagonists advanced to clinical trials in patients with mild-to-moderate AD, and were reported to improve scores on the Clinician's Interview-Based Impression of Change plus Caregiver Input (CIBIC+) and ADAS-cog as a stand-alone therapy; importantly, treatment caused minimal adverse events (Maher-Edwards et al., 2010; Maher-Edwards et al., 2011; Wilkinson et al., 2014). Despite their widely reported nootropic effects in animals, and potential to improve cognitive function in AD,

5-HT_6 receptor localization and physiology remains poorly understood.

5-HT_6 Receptor Effects on Synaptic Transmission: What Is Known and Unknown

Over a decade of literature attributes the memory enhancing effects of 5-HT_6 receptor antagonists to disinhibition – that is, an attenuation of inhibitory synaptic transmission that facilitates information flow through excitatory networks (Dawson et al., 2001; Woolley et al., 2004; Fone, 2008; Schechter et al., 2008; Codony et al., 2011; Ramirez, 2013). This idea comes from studies showing that systemic administration of a 5-HT_6 receptor agonist increased extracellular levels of GABA in rat frontal cortex, dorsal hippocampus, striatum, and amygdala, whereas systemic administration of a 5-HT_6 receptor antagonist has been shown to increase extracellular levels of glutamate and acetylcholine in those brain regions (Rogers and Hagan, 2001; Woolley et al., 2001; Stean et al., 2002; Foley et al., 2004; Hirst et al., 2006; Marcos et al., 2006; Schechter et al., 2008). Additionally, West et al., (2009) reported that 5-HT_6 receptor activation increased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) onto pyramidal cells in hippocampal area CA1 without affecting their amplitude (West et al., 2009). Importantly, these results, obtained while fast excitatory synaptic transmission was pharmacologically blocked, suggest that activation of 5-HT_6 receptors increased the excitability of inhibitory interneurons. Moreover, lesioning of serotonergic and cholinergic fibers originating in the brain stem failed to alter 5-HT_6 mRNA levels, implying that 5-HT_6 receptors do not function as autoreceptors or directly control cholinergic release (Gerard et al., 1996; Marcos et al., 2006).

Further support for the idea that 5-HT_6 receptor antagonists dampen inhibition comes from studies reporting 5-HT_6 receptor mRNA and protein colocalized with striatal GABAergic neurons (Ward and Dorsa, 1996; Marazziti et al., 2012). Additionally, 5-HT_6 receptors are GPCRs that are canonically positively coupled to adenylyl cyclase via G_s, such that 5-HT_6 receptor activation increases intracellular levels of cAMP, an effect thought to increase the overall excitability of neurons (Millan et al., 2008). Consistent with this idea, Bonsi et al. (2007) showed that blockade of 5-HT_6 receptors hyperpolarized the resting membrane potential of neurons expressing 5-HT_6 receptor mRNA in the striatum (Bonsi et al., 2007).

Controversy undermining the idea that 5-HT_6 receptor antagonists directly attenuate the activity of inhibitory interneurons comes from studies suggesting their expression on excitatory neurons. For example, a recent study by Helboe et al., (2015) reported 5-HT_6 receptor mRNA expression in the majority of pyramidal neurons and granule cells in the hippocampus, with much lower levels of expression in inhibitory interneurons (Helboe et al., 2015). Moreover, Wang et al. (2016) reported that a 5-HT_6 receptor agonist attenuated glutamate release from synaptosomes prepared from whole hippocampus homogenates in a $G_{i/o}$-dependent manner, suggesting the presence of 5-HT_6 receptors on presynaptic termini of excitatory neurons and unprecedented association with $G_{i/o}$ (Wang et al., 2016). Additionally, Bonzi et al. (2007) reported that 5-HT_6 receptor antagonists hyperpolarize striatal acetylcholinergic interneurons that were depolarized by bath application of serotonin (Bonsi et al., 2007), results that are at odds with studies reporting that ablation of cholinergic fibers had no effect on 5-HT_6 receptor mRNA expression (Gerard et al., 1996; Marcos et al., 2006). Moreover, results from the

study by Bonsi et al. (2007) also conflict with the disinhibition hypothesis, since acetylcholinergic activity is thought to promote excitability. Lastly, others have published examples of 5-HT_6 receptor expression on pyramidal neurons and astrocytes within the hippocampus (Woolley et al., 2004; Marazziti et al., 2013). However, these reports lack quantification of 5-HT_6 receptor expression patterns. Although these studies support the idea that 5-HT_6 receptors are expressed on both excitatory and inhibitory neurons, as well as glial cells, confirmation of their cell-type specific expression patterns is lacking, largely due to the unavailability of well characterized 5-HT_6 receptor-specific antibodies. Moreover, the effects of 5-HT_6 receptor activity on synaptic transmission in the DG, the entranceway into the hippocampus, are lacking altogether. Thus, Chapter 2 of this dissertation uses *in vitro* electrophysiology and immunohistochemistry to dissect 5-HT_6 receptor localization and effects on inhibitory synaptic transmission in the DG. We hypothesize that 5-HT_6 receptor activation increases inhibitory synaptic transmission onto DGCs and that 5-HT_6 receptor blockade decreases inhibitory synaptic transmission onto DGCs via their direct effects on inhibitory interneurons. To circumvent a lack of well-characterized antibodies, we obtained commercially available transgenic mice expressing a modified bacterial artificial chromosome (BAC) with an enhanced green fluorescent protein (EGFP) reporter upstream of the 5-HT_6 receptor gene (htr6-EGFP mice (Gong et al., 2003; GENSAT, 2016) Results from this study are expected to provide an anatomical and physiological blueprint for 5-HT_6 receptor antagonists' cognition enhancing effects, provide a platform for understanding serotonergic remodeling in disease, and direct future therapy discovery to treat memory deficits in neurological disorders such as AD and epilepsy.

Memory Deficits and Hyperexcitability in Disease:

A Double-Edge Sword

Another interesting caveat to the development of treatments for memory impairments is that many patients with cognitive dysfunction also exhibit seizures and aberrant hyperexcitability thought to contribute to cognitive dysfunction. For instance, depending on the study cited, 1.5-64% of AD patients have had at least 1 unprovoked seizure (Friedman et al., 2012). Moreover, at least half of patients with a dual-diagnosis of AD and epilepsy have been shown to exhibit nonconvulsive seizures that may go unnoticed (Vossel et al., 2013). And recently, a longitudinal study in AD patients with no prior history of seizures reported that 42% of patients (compared to 10.5% of age-matched controls) exhibited epileptiform activity, which are brief bursts of hypersynchronous discharges thought to impair cognitive function and facilitate disease progression (Palop et al., 2007; Sanchez et al., 2012; Kleen et al., 2013; Vossel et al., 2016). Additionally, adults and children with epilepsy report that memory deficits are amongst their greatest concerns (Thompson and Corcoran, 1992; Fisher et al., 2000), and individuals with intellectual disability and autism spectrum disorder also experience memory deficits and an increased risk of seizures compared to the general population (Cirillo and Seidman, 2003; Vicari et al., 2005; Boucher et al., 2012; Pauls et al., 2014; Jackson et al., 2015; Buckley and Holmes, 2016).

The occurrence of seizures and memory deficits in epilepsy, AD, and other neurological diseases presents a double-edged sword regarding pharmacological treatments: Many cognition-enhancing drugs have seizure liabilities, and many antiseizure drugs (ASDs) cause memory impairment. For example, 2 of the 5 currently

approved drug treatments for cognitive dysfunction in AD, Aricept (Donepezil) and Exelon (Rivastigmine), are listed on the World Health Organization's top ten drugs associated with convulsive adverse drug reactions (Kumlien and Lundberg, 2010). Moreover, memantine, the only approved treatment for cognitive dysfunction in AD that is not an acetylcholinesterase inhibitor, has been shown to worsen seizures in animal models of epilepsy (Loscher, 1998; Mares and Mikulecka, 2009), has been associated with new-onset seizures in AD (Peltz et al., 2005), and comes with a package warning for increased risk of seizures. Not only are seizures a dangerous side effect of the only approved treatment options available to AD patients, but seizures and hyperexcitability themselves, which can manifest as subclinical epileptiform activity that is difficult to detect, can impair memory and worsen disease progression (Palop et al., 2007; Bakker et al., 2012; Sanchez et al., 2012; Kleen et al., 2013). Thus, new treatments for memory deficits not only need to improve cognitive function, but they must do so without exacerbating the risk of seizures.

On the other hand, treating seizures in patients with memory impairment is hampered by the fact that many ASDs impair cognitive function. For example, ASDs such as carbamazepine, phenobarbital (PHB), phenytoin, topiramate (TPM), and valproate have all been shown to impair cognitive function in healthy individuals and patients with epilepsy (Meador et al., 1993; Meador et al., 1995; Meador, 2002; Lee et al., 2003; Meador et al., 2005; Salinsky et al., 2005; Hamed, 2009). Moreover, many ASD drugs have been shown to attenuate long-term synaptic plasticity at hippocampal synapses critical for new memory formation (West et al., 2014). However, dampening hyperexcitability with ASDs does not always have deleterious effects: second-generation

ASDs such as Levetiracetam (LEV) and TPM have been shown to attenuate seizures and improve cognitive function in animal models of AD that also exhibit spontaneous seizures (contrary to TPM's deleterious effects on cognition in humans) (Sanchez et al., 2012; Shi et al., 2013). Remarkably, LEV reduced seizures *and* improved cognitive dysfunction in patients with AD (Cumbo and Ligori, 2010; Bakker et al., 2012). PHB and lamotrigine were also effective at reducing seizures in AD patients, but had deleterious effects on patients' performance during the MMSE. Although these results are encouraging, and additional clinical trials testing the effects of LEV on seizures and cognitive function in AD are underway, new therapies that improve cognition without exacerbating seizures are needed. Ideally, new treatments should be both procognitive and anticonvulsant.

5-HT_6 Receptor Antagonists as a Potential Procognitive and Anticonvulsant Treatment

Interestingly, in addition to their nootropic effects, 5-HT_6 receptor antagonists also exhibit antiseizure properties. Several studies have reported that acute systemic treatment with a 5-HT_6 receptor antagonist increased seizure threshold in rats during the maximal electroshock test (Routledge et al., 2000; Stean et al., 2002; Hirst et al., 2006). Additionally, pretreatment with the 5-HT_6 receptor antagonist SB-399885 increased seizure latency and reduced seizure severity in rats treated with the proconvulsant compound pilocarpine (Wang et al., 2015). However, the effects of 5-HT_6 receptor antagonists in other acute seizure models remains unreported, and their effects on cognition and seizures in a transgenic model of AD, where seizures and hyperexcitability

are thought to contribute to disease progression, are also unreported. Understanding the effects of 5-HT_6 receptor antagonists on cognition and seizures in a transgenic model of AD is critical, since their effects may differ from those in otherwise healthy animals due to remodeling of serotonergic or other neurotransmitter systems affected by 5-HT_6 receptors in the disease state (Garcia-Alloza et al., 2004; Li et al., 2016). Furthermore, if 5-HT_6 receptor antagonists do affect cognition and seizures in a transgenic model of AD, these results would warrant a detailed investigation into 5-HT_6 receptor localization and physiology in transgenic models of AD, and perhaps guide their use in patients with AD.

Chapter 3 tests the hypothesis that 5-HT_6 receptor antagonists exhibit procognitive and anticonvulsant effects in J20 mice. J20 mice are a transgenic model of AD shown to exhibit cognitive dysfunction and spontaneous nonconvulsive seizures thought to mimic the subpopulation of patients with AD and epilepsy (Palop et al., 2007; Sanchez et al., 2012; Vossel et al., 2013; Vossel et al., 2016). We evaluated the procognitive and anticonvulsant effects of a 5-HT_6 receptor antagonist on cognition and seizures using a spatial pattern processing paradigm known as the metric task and the minimal clonic seizure threshold test, respectively. Chapter 3 also tests the hypothesis that 5-HT_6 receptor antagonists are anticonvulsant in 2 strains of naïve and otherwise healthy mice. Results from these studies are expected to inform the clinical use of 5-HT_6 receptor antagonists in patients with AD, particularly with respect to their effects on seizures, and provide justification for dissecting 5-HT_6 receptor physiology in animal models of AD.

Corneal Kindling in C57BL/6 Mice: A Bottom-Up Approach to Studying Memory Deficits in Epilepsy

Patients with epilepsy not only experience spontaneous seizures but also a host of cognitive comorbidities including memory dysfunction. Approximately 25% of all patients with TLE experience memory deficits (Hermann et al., 2006), and 70-80% of the pharmacoresistant population, which makes up about one third of the epilepsy population, exhibit declarative memory impairment (Helmstaedter, 2002). Moreover, surveys show that the majority of epilepsy patients report that memory deficits are their greatest concern (Thompson and Corcoran, 1992; Fisher et al., 2000). Unfortunately, there are currently no approved treatments for memory impairment associated with epilepsy. Accordingly, the 2014 National Institute of Neurological Disorders and Stroke (NINDS) Benchmarks for Epilepsy Research called for studies to better understand and limit adverse impacts of seizures on quality of life, including intellectual abilities, thus prompting detailed investigations of cognitive dysfunction and its associated pathophysiology in animal models of epilepsy.

The etiology of memory impairment in epilepsy is diverse and complex. Although there are a number of factors that contribute to cognitive dysfunction in epilepsy, cell death and hippocampal sclerosis (HS) are thought to play a critical role in memory dysfunction (Sutula et al., 1995; Kotloski et al., 2002). However, not all epilepsy patients demonstrating memory impairment have severe hippocampal damage (Aikia et al., 1995; Lah et al., 2014), and hippocampal cell loss with or without HS is not always associated with memory impairment (Castro et al., 2013; Schmidt et al., 2015). Moreover, associations between hippocampal volume loss and performance on memory tests are

highly variable (Aikia et al., 2001). These observations suggest, at least in some epilepsy patients, more subtle changes in hippocampal physiology may underlie memory dysfunction. Towards this end, animal models of epilepsy that exhibit memory deficits in the absence of overt neuronal loss may be useful in understanding the etiology of memory impairment in disease.

There are a number of animal models that produce the hallmark pathological features of epilepsy such as spontaneous seizures and cell death. For example, systemic administration of chemoconvulsants or direct electrical stimulation of limbic structures reliably produce spontaneous seizures and memory dysfunction in rodents but result in neuronal loss (Cossart et al., 2001; Hannesson et al., 2001; Sloviter et al., 2003; Harvey and Sloviter, 2005; Leung and Shen, 2006; Sloviter et al., 2006; Sun et al., 2007; Chauviere et al., 2009; Jinde et al., 2012). Although these models are useful because they recapitulate the pathophysiology found in many patients with epilepsy, developing treatments for memory impairments in epilepsy may be hampered in animal models that exhibit overt hippocampal damage. Support for this idea is highlighted by studies showing that patients with TLE or AD who exhibit severe cell loss are more likely to be pharmacoresistant and severely cognitively impaired (Helmstaedter, 2002; Connelly et al., 2005), leaving them few, if any, treatment options. While it is ideal to develop treatments for all patients who suffer from cognitive dysfunction, it may be a more manageable endeavor to first develop therapies in animal models that exhibit memory deficits as a result of more subtle hippocampal pathophysiology.

Towards this end, electrical kindling models that directly stimulate limbic brain regions exhibit hippocampal-dependent memory impairment in the absence of overt

hippocampal cell loss (Hannesson and Corcoran, 2000). This suggests that more subtle changes in hippocampal physiology underlie memory impairment in these models. However, electrical kindling models are constrained by laborious implantation surgeries that also cause a proinflammatory response in the brain. A noninvasive model of kindling that employs the chemoconvulsant pentylenetetrazol (PTZ) also produces hippocampal-dependent spatial memory impairment, but has been reported to produce hippocampal neuron loss (Pohle et al., 1997; Palizvan et al., 2001; Park et al., 2006; Mishra and Goel, 2012, 2013). Taken together, these studies highlight the need for a noninvasive model of epilepsy that exhibits memory deficits in the absent of overt hippocampal damage.

In a follow-up study to the report by Loewen et al. (2016) showing that corneal kindled CF1 mice exhibit no observable neuron loss in the hippocampus (Loewen et al., 2016), Chapter 4 of this dissertation tested corneal kindled C57BL/6 mice for cognitive dysfunction in the metric task, a spatial pattern processing paradigm thought to rely on proper DG function (Ennaceur and Delacour, 1988; Lee et al., 2005; Goodrich-Hunsaker et al., 2008; Ennaceur, 2010). The corneal kindling model of network hyperexcitability in mice has been validated as a rapid screening model of focal seizures that secondarily generalize and has been validated as a useful tool in the discovery of novel ASDs, in part, because of its noninvasive, cost-effective methodology (Matagne and Klitgaard, 1998; Potschka and Loscher, 1999; Rowley and White, 2010). Upon discovery of spatial pattern processing deficits in corneal kindled mice, we used immunohistochemistry and *in vitro* hippocampal brain slice electrophysiology to test the hypothesis that corneal kindled mice exhibit altered dentate gyrus-associated synaptic transmission and plasticity in the absence of neuron loss. Results from this study are expected to inform the etiology of

memory deficits in epilepsy and perhaps provide a useful model for the development of treatments that ameliorate epilepsy-associated cognitive dysfunction.

The Dentate Gyrus and Its Role in Learning and Memory

Almost all of the experiments in this dissertation are focused on the physiology and function of the DG. The DG is a subregion in the hippocampus, an evolutionarily conserved bilateral temporal lobe structure that is critical for new memory formation (Squire, 1992). Anatomically, the hippocampus can be divided into 3 interconnected subareas: the DG, CA3, and CA1. These 3 regions form the trisynaptic loop, beginning with perforant-path projections from the entorhinal cortex to DG granule cells (DGCs), which then project to pyramidal neurons in hippocampal area CA3; CA3 then projects to CA1. Dissecting the physiology and function of the hippocampus and its individual subregions in health and disease is a cornerstone of neuroscience research, in part, because it is expected to lay the foundation for the understanding of, and amelioration of, memory impairment in disease.

Studies investigating the individual contribution of hippocampal subregions in rodents show that the DG and CA3 play a critical role in the rapid acquisition of new spatial memories, whereas hippocampal area CA1 is thought to give temporal context to mnemonic representations (Kesner et al., 2004; Barbosa et al., 2012). More specifically, the DG is thought to play a critical role in fine scale pattern separation (Lassalle et al., 2000; Lee et al., 2005; Jerman et al., 2006; Morris et al., 2012), which is the ability to encode and separate spatially similar events into distinct representations. The DG performs this complex function, in part, by maintaining low resting membrane potentials

and low levels of spontaneous activity in DGCs as a result of robust afferent inhibition from inhibitory interneurons that gate information flow into the hippocampus (Coulter and Carlson, 2007; Hsu, 2007). These properties allow DGCs to orthogonalize patterns of information that have a high degree of overlap, and thus create metric spatial representations out of sensory information coming from entorhinal cortex.

Rationale for studying 5-HT_6 receptor physiology and function in the DG stems from preliminary data generated in our lab (presented in Chapter 2) showing that the 5-HT_6 receptor agonist WAY-208466 attenuated the amplitude of field excitatory postsynaptic potentials (fEPSPs) generated at perforant path-DG granule cell (DGC) synapses in hippocampal brain slices. Notably, this effect was not observed when tested on fEPSPs generated at Schafer Collateral-pyramidal neuron synapses in hippocampal area CA1 (West et al., 2009), intimating that 5-HT_6 receptors may play a critical role in DG function. Moreover, other studies suggest that 5-HT_6 receptor antagonists likely exert their memory enhancing effects, in part, by attenuating inhibition and thus increasing information flow through excitatory networks. Being that the DG harbors at least 5 inhibitory interneurons subtypes that gate information flow into the hippocampus (Hosp et al., 2014), the DG might be an ideal anatomical location for 5-HT_6 receptor antagonists to exert their nootropic effects through disinhibition, and perhaps via a select subpopulation of inhibitory interneurons.

Despite an extensive literature delineating the nootropic effects of 5-HT_6 receptor antagonists on cognitive function, their effects during a pattern separation task thought to rely on DG function have, to my knowledge, never been published. Therefore, in addition to testing their effects on synaptic transmission in the DG, we wanted to test 5-HT_6

receptor antagonists in a DG-mediated spatial pattern processing task that relies on rodents' natural tendency to explore changes in the distance between 2 objects – the "metric task" (Ennaceur and Delacour, 1988; Lee et al., 2005; Goodrich-Hunsaker et al., 2008; Ennaceur, 2010). More specifically, we wanted to utilize the metric task to evaluate J20 (AD) mice for DG-associated cognitive dysfunction, as others have reported abnormalities in synaptic transmission and synaptic plasticity in the DG of J20 mice (Palop et al., 2007). Lastly, we also evaluated whether corneal kindled C57BL/6 mice exhibited DG-dependent memory deficits in the metric task, and dissect whether they exhibited DG-associated abnormalities in synaptic transmission and plasticity.

Dissertation Overview

In summary, the research in this dissertation adopted a top-down and bottom-up approach to studying memory enhancement and memory deficits related to DG physiology and function. See Table 1.1 for a summary of the hypotheses tested in this dissertation. Chapter 2 dissects 5-HT_6 receptor localization and physiology in the DG, and Chapter 3 focused on the behavioral pharmacology to 5-HT_6 receptors antagonists, investigating their effect on spatial pattern processing in transgenic model of AD. Moreover, studies in Chapter 3 also tested the effects of 5-HT_6 receptor antagonists on seizure threshold in J20 mice, as well as naïve and otherwise healthy mice. Lastly, Chapter 4 investigated DG-associated spatial pattern processing in corneal kindled mice, and related pathophysiology in the DG that occurred in the absence of overt neuron loss. Results from these studies are expected to inform the mechanistic basis of memory enhancement and memory impairments, particularly as they relate to DG physiology and

function, and direct the future treatment of memory deficits in diseases such as AD and epilepsy.

Table 1.1

Summary of the hypotheses tested in this dissertation by chapter.

Chapter	Hypotheses Tested
2	5-HT_6 receptor activation increases inhibitory synaptic transmission onto DGCs, whereas 5-HT_6 receptor blockade decreases inhibitory synaptic transmission onto DGCs, via their direct effects on inhibitory interneurons.
3	5-HT_6 receptor antagonists exhibit procognitive and anticonvulsant effects in J20 mice. 5-HT_6 receptor antagonists exhibit anticonvulsant effects in naïve mice.
4	Corneal kindled mice exhibit dentate gyrus-associated spatial pattern processing deficits and altered synaptic transmission and plasticity in the absence of neuron loss.

References

Aikia M, Kalviainen R, Riekkinen PJ (1995) Verbal learning and memory in newly diagnosed partial epilepsy. Epilepsy Res 22:157-164.

Aikia M, Salmenpera T, Partanen K, Kalviainen R (2001) Verbal memory in newly diagnosed patients and patients with chronic left temporal lobe epilepsy. Epilepsy Behav 2:20-27.

Andel R, Hyer K, Slack A (2007) Risk factors for nursing home placement in older adults with and without dementia. J Aging Health 19:213-228.

Aznar S, Hervig Mel S (2016) The 5-HT2A serotonin receptor in executive function: Implications for neuropsychiatric and neurodegenerative diseases. Neurosci Biobehav Rev 64:63-82.

Bagdy G, Kecskemeti V, Riba P, Jakus R (2007) Serotonin and epilepsy. J Neurochem 100:857-873.

Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, Yassa MA, Bassett SS, Shelton AL, Gallagher M (2012) Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron 74:467-474.

Barbosa FF, Pontes IM, Ribeiro S, Ribeiro AM, Silva RH (2012) Differential roles of the dorsal hippocampal regions in the acquisition of spatial and temporal aspects of episodic-like memory. Behav Brain Res 232:269-277.

Benhamu B, Martin-Fontecha M, Vazquez-Villa H, Pardo L, Lopez-Rodriguez ML (2014) Serotonin 5-HT receptor antagonists for the treatment of cognitive deficiency in Alzheimer's disease. J Med Chem 57:7160-7181.

Berg AT (2011) Epilepsy, cognition, and behavior: The clinical picture. Epilepsia 52:7-12.

Berg AT, Mathern GW, Bronen RA, Fulbright RK, DiMario F, Testa FM, Levy SR (2009) Frequency, prognosis and surgical treatment of structural abnormalities seen with magnetic resonance imaging in childhood epilepsy. Brain 132:2785-2797.

Berger M, Gray JA, Roth BL (2009) The expanded biology of serotonin. Annu Rev Med 60:355-366.

Bonsi P, Cuomo D, Ding J, Sciamanna G, Ulrich S, Tscherter A, Bernardi G, Surmeier DJ, Pisani A (2007) Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6, and 5-HT7 serotonin receptors: implications for extrapyramidal side effects of serotonin reuptake inhibitors. Neuropsychopharmacology 32:1840-1854.

Boucher J, Mayes A, Bigham S (2012) Memory in autistic spectrum disorder. Psychol

Bull 138:458-496.

Buckley AW, Holmes GL (2016) Epilepsy and autism. Cold Spring Harb Perspect Med 6:a022749.

Castro LH, Silva LC, Adda CC, Banaskiwitz NH, Xavier AB, Jorge CL, Valerio RM, Nitrini R (2013) Low prevalence but high specificity of material-specific memory impairment in epilepsy associated with hippocampal sclerosis. Epilepsia 54:1735-1742.

Chauviere L, Rafrafi N, Thinus-Blanc C, Bartolomei F, Esclapez M, Bernard C (2009) Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. J Neurosci 29:5402-5410.

Cirillo MA, Seidman LJ (2003) Verbal declarative memory dysfunction in schizophrenia: from clinical assessment to genetics and brain mechanisms. Neuropsychol Rev 13:43-77.

Codony X, Vela JM, Ramirez MJ (2011) 5-HT(6) receptor and cognition. Curr Opin Pharmacol 11:94-100.

Connelly PJ, Prentice NP, Fowler KG (2005) Predicting the outcome of cholinesterase inhibitor treatment in Alzheimer's disease. J Neurol Neurosurg Psychiatry 76:320-324.

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben-Ari Y, Esclapez M, Bernard C (2001) Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nat Neurosci 4:52-62.

Coulter DA, Carlson GC (2007) Functional regulation of the dentate gyrus by GABA-mediated inhibition. Prog Brain Res 163:235-243.

Cumbo E, Ligori LD (2010) Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. Epilepsy Behav 17:461-466.

Dawson LA, Nguyen HQ, Li P (2001) The 5-HT(6) receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. Neuropsychopharmacology 25:662-668.

Duhr F, Deleris P, Raynaud F, Seveno M, Morisset-Lopez S, Mannoury la Cour C, Millan MJ, Bockaert J, Marin P, Chaumont-Dubel S (2014) Cdk5 induces constitutive activation of 5-HT6 receptors to promote neurite growth. Nat Chem Biol 10:590-597.

Ennaceur A (2010) One-trial object recognition in rats and mice: methodological and theoretical issues. Behav Brain Res 215:244-254.

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res 31:47-59.

Fisher RS, Vickrey BG, Gibson P, Hermann B, Penovich P, Scherer A, Walker S (2000) The impact of epilepsy from the patient's perspective I. Descriptions and subjective perceptions. Epilepsy Res 41:39-51.

Foley AG, Murphy KJ, Hirst WD, Gallagher HC, Hagan JJ, Upton N, Walsh FS, Regan CM (2004) The 5-HT(6) receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. Neuropsychopharmacology 29:93-100.

Fone KC (2008) An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. Neuropharmacology 55:1015-1022.

Friedman D, Honig LS, Scarmeas N (2012) Seizures and epilepsy in Alzheimer's disease. CNS Neurosci Ther 18:285-294.

Frisoni GB, Fox NC, Jack CR, Jr., Scheltens P, Thompson PM (2010) The clinical use of structural MRI in Alzheimer disease. Nat Rev Neurol 6:67-77.

Garcia-Alloza M, Hirst WD, Chen CP, Lasheras B, Francis PT, Ramirez MJ (2004) Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer's disease. Neuropsychopharmacology 29:410-416.

GENSAT Gene Expression Nervous System Atlas. Available at: http://gensat.org/index.html [Accessed: October 10, 2016].

Gerard C, el Mestikawy S, Lebrand C, Adrien J, Ruat M, Traiffort E, Hamon M, Martres MP (1996) Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. Synapse 23:164-173.

Gerard C, Martres MP, Lefevre K, Miquel MC, Verge D, Lanfumey L, Doucet E, Hamon M, el Mestikawy S (1997) Immuno-localization of serotonin 5-HT6 receptor-like material in the rat central nervous system. Brain Res 746:207-219.

Gold CA, Budson AE (2008) Memory loss in Alzheimer's disease: implications for development of therapeutics. Expert Rev Neurother 8:1879-1891.

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature 425:917-925.

Goodrich-Hunsaker NJ, Hunsaker MR, Kesner RP (2008) The interactions and dissociations of the dorsal hippocampus subregions: how the dentate gyrus, CA3, and CA1 process spatial information. Behav Neurosci 122:16-26.

Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER (1992) Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice.

Science 258:1903-1910.

Hamed SA (2009) The aspects and mechanisms of cognitive alterations in epilepsy: the role of antiepileptic medications. CNS Neurosci Ther 15:134-156.

Hannesson DK, Corcoran ME (2000) The mnemonic effects of kindling. Neurosci Biobehav Rev 24:725-751.

Hannesson DK, Mohapel P, Corcoran ME (2001) Dorsal hippocampal kindling selectively impairs spatial learning/short-term memory. Hippocampus 11:275-286.

Harvey BD, Sloviter RS (2005) Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. J Comp Neurol 488:442-463.

Helmstaedter C (2002) Effects of chronic epilepsy on declarative memory systems. Prog Brain Res 135:439-453.

Hermann BP, Seidenberg M, Dow C, Jones J, Rutecki P, Bhattacharya A, Bell B (2006) Cognitive prognosis in chronic temporal lobe epilepsy. Ann Neurol 60:80-87.

Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Medhurst AD (2003) Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol 64:1295-1308.

Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM, Riley G, Smith DR, Bartlett S, Heidbreder CA, Atkins AR, Lacroix LP, Dawson LA, Foley AG, Regan CM, Upton N (2006) SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol 553:109-119.

Hosp JA, Struber M, Yanagawa Y, Obata K, Vida I, Jonas P, Bartos M (2014) Morpho-physiological criteria divide dentate gyrus interneurons into classes. Hippocampus 24:189-203.

Hsu D (2007) The dentate gyrus as a filter or gate: a look back and a look ahead. Prog Brain Res 163:601-613.

Jackson CF, Makin SM, Marson AG, Kerr M (2015) Pharmacological interventions for epilepsy in people with intellectual disabilities. Cochrane Database Syst Rev:CD005399.

Jerman T, Kesner RP, Hunsaker MR (2006) Disconnection analysis of CA3 and DG in mediating encoding but not retrieval in a spatial maze learning task. Learn Mem 13:458-464.

Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K (2012) Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron 76:1189-1200.

Kesner RP, Lee I, Gilbert P (2004) A behavioral assessment of hippocampal function based on a subregional analysis. Rev Neurosci 15:333-351.

King MV, Marsden CA, Fone KC (2008) A role for the 5-HT(1A), 5-HT4 and 5-HT6 receptors in learning and memory. Trends Pharmacol Sci 29:482-492.

King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KC (2004) 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation--an effect sensitive to NMDA receptor antagonism. Neuropharmacology 47:195-204.

Kleen JK, Scott RC, Holmes GL, Roberts DW, Rundle MM, Testorf M, Lenck-Santini PP, Jobst BC (2013) Hippocampal interictal epileptiform activity disrupts cognition in humans. Neurology 81:18-24.

Kohen R, Fashingbauer LA, Heidmann DE, Guthrie CR, Hamblin MW (2001) Cloning of the mouse 5-HT6 serotonin receptor and mutagenesis studies of the third cytoplasmic loop. Brain Res Mol Brain Res 90:110-117.

Kohen R, Metcalf MA, Khan N, Druck T, Huebner K, Lachowicz JE, Meltzer HY, Sibley DR, Roth BL, Hamblin MW (1996) Cloning, characterization, and chromosomal localization of a human 5-HT6 serotonin receptor. J Neurochem 66:47-56.

Korenberg JR, West, P. J., Wilcox, K. S., Smith, G. K. & Kesner, R. P. Paradigm Shift for the treatment of cognitive deficits in Down syndrome: A novel drug treatment rescues hippocampal deficits in the Ts65Dn mouse. Presented at the 63rd Annual Meeting of the ASHG, October 23, 2013 in Boston, MA.

Kotloski R, Lynch M, Lauersdorf S, Sutula T (2002) Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. Prog Brain Res 135:95-110.

Kumlien E, Lundberg PO (2010) Seizure risk associated with neuroactive drugs: data from the WHO adverse drug reactions database. Seizure 19:69-73.

Lah S, Mohamed A, Thayer Z, Miller L, Diamond K (2014) Accelerated long-term forgetting of verbal information in unilateral temporal lobe epilepsy: Is it related to structural hippocampal abnormalities and/or incomplete learning? J Clin Exp Neuropsychol 36:158-169.

Lassalle JM, Bataille T, Halley H (2000) Reversible inactivation of the hippocampal mossy fiber synapses in mice impairs spatial learning, but neither consolidation nor memory retrieval, in the Morris navigation task. Neurobiol Learn Mem 73:243-257.

Lee I, Hunsaker MR, Kesner RP (2005) The role of hippocampal subregions in detecting spatial novelty. Behav Neurosci 119:145-153.

Lee S, Sziklas V, Andermann F, Farnham S, Risse G, Gustafson M, Gates J, Penovich P, Al-Asmi A, Dubeau F, Jones-Gotman M (2003) The effects of adjunctive topiramate on cognitive function in patients with epilepsy. Epilepsia 44:339-347.

Leung LS, Shen B (2006) Hippocampal CA1 kindling but not long-term potentiation disrupts spatial memory performance. Learn Mem 13:18-26.

Li Y, Sun H, Chen Z, Xu H, Bu G, Zheng H (2016) Implications of GABAergic neurotransmission in Alzheimer's disease. Front Aging Neurosci 8:31.

Loewen JL, Barker-Haliski ML, Dahle EJ, White HS, Wilcox KS (2016) Neuronal injury, gliosis, and glial proliferation in two models of temporal lobe epilepsy. J Neuropathol Exp Neurol 75:366-378.

Loscher W (1998) Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy. Prog Neurobiol 54:721-741.

Maher-Edwards G, Dixon R, Hunter J, Gold M, Hopton G, Jacobs G, Hunter J, Williams P (2011) SB-742457 and donepezil in Alzheimer disease: a randomized, placebo-controlled study. Int J Geriatr Psychiatry 26:536-544.

Maher-Edwards G, Zvartau-Hind M, Hunter AJ, Gold M, Hopton G, Jacobs G, Davy M, Williams P (2010) Double-blind, controlled phase II study of a 5-HT6 receptor antagonist, SB-742457, in Alzheimer's disease. Curr Alzheimer Res 7:374-385.

Marazziti D, Baroni S, Pirone A, Giannaccini G, Betti L, Schmid L, Vatteroni E, Palego L, Borsini F, Bordi F, Piano I, Gargini C, Castagna M, Catena-Dell'osso M, Lucacchini A (2012) Distribution of serotonin receptor of type 6 (5-HT(6)) in human brain post-mortem. A pharmacology, autoradiography and immunohistochemistry study. Neurochem Res 37:920-927.

Marazziti D, Baroni S, Pirone A, Giannaccini G, Betti L, Testa G, Schmid L, Palego L, Borsini F, Bordi F, Piano I, Gargini C, Castagna M, Catena-Dell'osso M, Lucacchini A (2013) Serotonin receptor of type 6 (5-HT6) in human prefrontal cortex and hippocampus post-mortem: an immunohistochemical and immunofluorescence study. Neurochem Int 62:182-188.

Marcos B, Gil-Bea FJ, Hirst WD, Garcia-Alloza M, Ramirez MJ (2006) Lack of localization of 5-HT6 receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT6 receptor-mediated acetylcholine release. Eur J Neurosci 24:1299-1306.

Mares P, Mikulecka A (2009) Different effects of two N-methyl-D-aspartate receptor antagonists on seizures, spontaneous behavior, and motor performance in immature rats. Epilepsy Behav 14:32-39.

Matagne A, Klitgaard H (1998) Validation of corneally kindled mice: a sensitive screening model for partial epilepsy in man. Epilepsy Res 31:59-71.

Mawe GM, Hoffman JM (2013) Serotonin signalling in the gut--functions, dysfunctions and therapeutic targets. Nat Rev Gastroenterol Hepatol 10:473-486.

McCorvy JD, Roth BL (2015) Structure and function of serotonin G protein-coupled receptors. Pharmacol Ther 150:129-142.

McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack CR, Jr., Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R, Mohs RC, Morris JC, Rossor MN, Scheltens P, Carrillo MC, Thies B, Weintraub S, Phelps CH (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:263-269.

Meador KJ (2002) Cognitive outcomes and predictive factors in epilepsy. Neurology 58:S21-26.

Meador KJ, Loring DW, Abney OL, Allen ME, Moore EE, Zamrini EY, King DW (1993) Effects of carbamazepine and phenytoin on EEG and memory in healthy adults. Epilepsia 34:153-157.

Meador KJ, Loring DW, Moore EE, Thompson WO, Nichols ME, Oberzan RE, Durkin MW, Gallagher BB, King DW (1995) Comparative cognitive effects of phenobarbital, phenytoin, and valproate in healthy adults. Neurology 45:1494-1499.

Meador KJ, Loring DW, Vahle VJ, Ray PG, Werz MA, Fessler AJ, Ogrocki P, Schoenberg MR, Miller JM, Kustra RP (2005) Cognitive and behavioral effects of lamotrigine and topiramate in healthy volunteers. Neurology 64:2108-2114.

Meffre J, Chaumont-Dubel S, Mannoury la Cour C, Loiseau F, Watson DJ, Dekeyne A, Seveno M, Rivet JM, Gaven F, Deleris P, Herve D, Fone KC, Bockaert J, Millan MJ, Marin P (2012) 5-HT(6) receptor recruitment of mTOR as a mechanism for perturbed cognition in schizophrenia. EMBO Mol Med 4:1043-1056.

Meneses A, Perez-Garcia G, Ponce-Lopez T, Castillo C (2011) 5-HT6 receptor memory and amnesia: behavioral pharmacology--learning and memory processes. Int Rev Neurobiol 96:27-47.

Millan MJ, Marin P, Bockaert J, Mannoury la Cour C (2008) Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. Trends Pharmacol Sci 29:454-464.

Mishra A, Goel RK (2012) Age dependent learning and memory deficit in Pentylenetetrazol kindled mice. Eur J Pharmacol 674:315-320.

Mishra A, Goel RK (2013) Psychoneurochemical investigations to reveal neurobiology

of memory deficit in epilepsy. Neurochem Res 38:2503-2515.

Mitchell ES, Hoplight BJ, Lear SP, Neumaier JF (2006) BGC20-761, a novel tryptamine analog, enhances memory consolidation and reverses scopolamine-induced memory deficit in social and visuospatial memory tasks through a 5-HT6 receptor-mediated mechanism. Neuropharmacology 50:412-420.

Mnie-Filali O, Faure C, Lambas-Senas L, El Mansari M, Belblidia H, Gondard E, Etievant A, Scarna H, Didier A, Berod A, Blier P, Haddjeri N (2011) Pharmacological blockade of 5-HT7 receptors as a putative fast acting antidepressant strategy. Neuropsychopharmacology 36:1275-1288.

Mnie-Filali O, Lambas-Senas L, Zimmer L, Haddjeri N (2007) 5-HT7 receptor antagonists as a new class of antidepressants. Drug News Perspect 20:613-618.

Monsma FJ, Jr., Shen Y, Ward RP, Hamblin MW, Sibley DR (1993) Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol Pharmacol 43:320-327.

Morris AM, Churchwell JC, Kesner RP, Gilbert PE (2012) Selective lesions of the dentate gyrus produce disruptions in place learning for adjacent spatial locations. Neurobiol Learn Mem 97:326-331.

Mosinska P, Salaga M, Fichna J (2016) Novel investigational drugs for constipation-predominant irritable bowel syndrome: a review. Expert Opin Investig Drugs 25:275-286.

Nygaard HB (2013) Current and emerging therapies for Alzheimer's disease. Clin Ther 35:1480-1489.

Palizvan MR, Fathollahi Y, Semnanian S, Hajezadeh S, Mirnajafizadh J (2001) Differential effects of pentylenetetrazol-kindling on long-term potentiation of population excitatory postsynaptic potentials and population spikes in the CA1 region of rat hippocampus. Brain Res 898:82-90.

Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55:697-711.

Panczyk K, Golda S, Waszkielewicz A, Zelaszczyk D, Gunia-Krzyzak A, Marona H (2015) Serotonergic system and its role in epilepsy and neuropathic pain treatment: a review based on receptor ligands. Curr Pharm Des 21:1723-1740.

Park JH, Cho H, Kim H, Kim K (2006) Repeated brief epileptic seizures by pentylenetetrazole cause neurodegeneration and promote neurogenesis in discrete brain regions of freely moving adult rats. Neuroscience 140:673-684.

Pauls F, Petermann F, Lepach AC (2014) Episodic memory and executive functioning in currently depressed patients compared to healthy controls. Cogn Emot:1-18.

Peltz G, Pacific DM, Noviasky JA, Shatla A, Mehalic T (2005) Seizures associated with memantine use. Am J Health Syst Pharm 62:420-421.

Pohle W, Becker A, Grecksch G, Juhre A, Willenberg A (1997) Piracetam prevents pentylenetetrazol kindling-induced neuronal loss and learning deficits. Seizure 6:467-474.

Pollack MH (2005) Comorbid anxiety and depression. J Clin Psychiatry 66 Suppl 8:22-29.

Potschka H, Loscher W (1999) Corneal kindling in mice: behavioral and pharmacological differences to conventional kindling. Epilepsy Res 37:109-120.

Raab-Graham KF, Haddick PC, Jan YN, Jan LY (2006) Activity- and mTOR-dependent suppression of Kv1.1 channel mRNA translation in dendrites. Science 314:144-148.

Raina P, Santaguida P, Ismaila A, Patterson C, Cowan D, Levine M, Booker L, Oremus M (2008) Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. Ann Intern Med 148:379-397.

Ramirez MJ (2013) 5-HT6 receptors and Alzheimer's disease. Alzheimers Res Ther 5:15.

Riccio O, Potter G, Walzer C, Vallet P, Szabo G, Vutskits L, Kiss JZ, Dayer AG (2009) Excess of serotonin affects embryonic interneuron migration through activation of the serotonin receptor 6. Mol Psychiatry 14:280-290.

Roberts JC, Reavill C, East SZ, Harrison PJ, Patel S, Routledge C, Leslie RA (2002) The distribution of 5-HT(6) receptors in rat brain: an autoradiographic binding study using the radiolabelled 5-HT(6) receptor antagonist [(125)I]SB-258585. Brain Res 934:49-57.

Rogers DC, Hagan JJ (2001) 5-HT6 receptor antagonists enhance retention of a water maze task in the rat. Psychopharmacology (Berl) 158:114-119.

Roth BL, Craigo SC, Choudhary MS, Uluer A, Monsma FJ, Jr., Shen Y, Meltzer HY, Sibley DR (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J Pharmacol Exp Ther 268:1403-1410.

Routledge C, Bromidge SM, Moss SF, Price GW, Hirst W, Newman H, Riley G, Gager T, Stean T, Upton N, Clarke SE, Brown AM, Middlemiss DN (2000) Characterization of SB-271046: a potent, selective and orally active 5-HT(6) receptor antagonist. Br J Pharmacol 130:1606-1612.

Rowley NM, White HS (2010) Comparative anticonvulsant efficacy in the corneal kindled mouse model of partial epilepsy: Correlation with other seizure and epilepsy models. Epilepsy Res 92:163-169.

Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R, Schwartz JC (1993) A novel rat serotonin (5-HT6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. Biochem Biophys Res Commun 193:268-276.

Rzezak P, Valente KD, Duchowny MS (2014) Temporal lobe epilepsy in children: executive and mnestic impairments. Epilepsy Behav 31:117-122.

Salinsky MC, Storzbach D, Spencer DC, Oken BS, Landry T, Dodrill CB (2005) Effects of topiramate and gabapentin on cognitive abilities in healthy volunteers. Neurology 64:792-798.

Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu GQ, Palop JJ, Mucke L (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc Natl Acad Sci U S A 109:E2895-2903.

Schechter LE, Lin Q, Smith DL, Zhang G, Shan Q, Platt B, Brandt MR, Dawson LA, Cole D, Bernotas R, Robichaud A, Rosenzweig-Lipson S, Beyer CE (2008) Neuropharmacological profile of novel and selective 5-HT6 receptor agonists: WAY-181187 and WAY-208466. Neuropsychopharmacology 33:1323-1335.

Schmidt CS, Lassonde M, Gagnon L, Sauerwein CH, Carmant L, Major P, Paquette N, Lepore F, Gallagher A (2015) Neuropsychological functioning in children with temporal lobe epilepsy and hippocampal atrophy without mesial temporal sclerosis: a distinct clinical entity? Epilepsy Behav 44:17-22.

Schreiber R, Vivian J, Hedley L, Szczepanski K, Secchi RL, Zuzow M, van Laarhoven S, Moreau JL, Martin JR, Sik A, Blokland A (2007) Effects of the novel 5-HT(6) receptor antagonist RO4368554 in rat models for cognition and sensorimotor gating. Eur Neuropsychopharmacol 17:277-288.

Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81:741-766.

Shi JQ, Wang BR, Tian YY, Xu J, Gao L, Zhao SL, Jiang T, Xie HG, Zhang YD (2013) Antiepileptics topiramate and levetiracetam alleviate behavioral deficits and reduce neuropathology in APPswe/PS1dE9 transgenic mice. CNS Neurosci Ther 19:871-881.

Skodzik T, Holling H, Pedersen A (2013) Long-term memory performance in adult ADHD: a meta-analysis. J Atten Disord [Epub ahead of print].

Sloviter RS, Zappone CA, Harvey BD, Bumanglag AV, Bender RA, Frotscher M (2003) "Dormant basket cell" hypothesis revisited: relative vulnerabilities of dentate gyrus

mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. J Comp Neurol 459:44-76.

Sloviter RS, Zappone CA, Harvey BD, Frotscher M (2006) Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyper-inhibition in chronically epileptic rats. J Comp Neurol 494:944-960.

Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev 99:195-231.

Stean TO, Hirst WD, Thomas DR, Price GW, Rogers D, Riley G, Bromidge SM, Serafinowska HT, Smith DR, Bartlett S, Deeks N, Duxon M, Upton N (2002) Pharmacological profile of SB-357134: a potent, selective, brain penetrant, and orally active 5-HT(6) receptor antagonist. Pharmacol Biochem Behav 71:645-654.

Sun C, Mtchedlishvili Z, Bertram EH, Erisir A, Kapur J (2007) Selective loss of dentate hilar interneurons contributes to reduced synaptic inhibition of granule cells in an electrical stimulation-based animal model of temporal lobe epilepsy. J Comp Neurol 500:876-893.

Sutula T, Lauersdorf S, Lynch M, Jurgella C, Woodard A (1995) Deficits in radial arm maze performance in kindled rats: evidence for long-lasting memory dysfunction induced by repeated brief seizures. J Neurosci 15:8295-8301.

Swiech L, Perycz M, Malik A, Jaworski J (2008) Role of mTOR in physiology and pathology of the nervous system. Biochim Biophys Acta 1784:116-132.

Thompson PJ, Corcoran R (1992) Everyday memory failures in people with epilepsy. Epilepsia 33 Suppl 6:S18-20.

Vicari S, Bellucci S, Carlesimo GA (2005) Visual and spatial long-term memory: differential pattern of impairments in Williams and Down syndromes. Dev Med Child Neurol 47:305-311.

Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, Hegde M, Cornes SB, Henry ML, Nelson AB, Seeley WW, Geschwind MD, Gorno-Tempini ML, Shih T, Kirsch HE, Garcia PA, Miller BL, Mucke L (2013) Seizures and epileptiform activity in the early stages of Alzheimer disease. JAMA Neurol 70:1158-1166.

Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, Darwish SM, Van Berlo V, Barnes DE, Mantle M, Karydas AM, Coppola G, Roberson ED, Miller BL, Garcia PA, Kirsch HE, Mucke L, Nagarajan SS (2016) Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. Ann Neurol 80:858-870.

Wang HY, Lu CW, Lin TY, Kuo JR, Wang SJ (2016) WAY208466 inhibits glutamate release at hippocampal nerve terminals. Eur J Pharmacol 781:117-127.

Wang L, Lv Y, Deng W, Peng X, Xiao Z, Xi Z, Chen G, Wang X (2015) 5-HT6 receptor recruitment of mTOR modulates seizure activity in epilepsy. Mol Neurobiol 51:1292-1299.

Ward RP, Dorsa DM (1996) Colocalization of serotonin receptor subtypes 5-HT2A, 5-HT2C, and 5-HT6 with neuropeptides in rat striatum. J Comp Neurol 370:405-414.

Wesolowska A (2010) Potential role of the 5-HT6 receptor in depression and anxiety: an overview of preclinical data. Pharmacol Rep 62:564-577.

West PJ, Marcy VR, Marino MJ, Schaffhauser H (2009) Activation of the 5-HT(6) receptor attenuates long-term potentiation and facilitates GABAergic neurotransmission in rat hippocampus. Neuroscience 164:692-701.

West PJ, Saunders GW, Remigio GJ, Wilcox KS, White HS (2014) Antiseizure drugs differentially modulate theta-burst induced long-term potentiation in C57BL/6 mice. Epilepsia 55:214-223.

Wilkinson D, Windfeld K, Colding-Jorgensen E (2014) Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Neurol 13:1092-1099.

Woolley ML, Bentley JC, Sleight AJ, Marsden CA, Fone KC (2001) A role for 5-ht6 receptors in retention of spatial learning in the Morris water maze. Neuropharmacology 41:210-219.

Woolley ML, Marsden CA, Fone KC (2004) 5-ht6 receptors. Curr Drug Targets CNS Neurol Disord 3:59-79.

Yang C, Xiao S (2015) New developments of clinical trial in immunotherapy for Alzheimer's disease. Curr Pharm Biotechnol 16:484-491.

Yun HM, Kim S, Kim HJ, Kostenis E, Kim JI, Seong JY, Baik JH, Rhim H (2007) The novel cellular mechanism of human 5-HT6 receptor through an interaction with Fyn. J Biol Chem 282:5496-5505.

CHAPTER 2

5-HT_6 RECEPTOR LOCALIZATION AND EFFECTS ON INHIBITORYSYNAPTIC TRANSMISION IN THE DENTATE GYRUS

Abstract

Current treatments for memory deficits in Alzheimer's disease (AD) are few in number, mechanistically homogenous, and only marginally effective. Thus, new treatments are needed. One approach is to first obtain a mechanistic understanding of existing treatments, including those in development, in order to "fine-tune" their targeted effects or discover new therapeutic approaches. Towards this end, 5-HT_6 receptor antagonists have been widely reported to produce nootropic effects and are thought to exert their memory enhancing effects by reducing inhibition. However, 5-HT_6 receptor cell-type specific expression patterns remain poorly understood, and their effects on inhibition in the dentate gyrus (DG), a brain area critical for learning and memory, are unreported. Therefore, we tested the hypothesis that 5-HT_6 receptor activation increases inhibitory synaptic transmission onto DG granule cells (DGCs), whereas 5-HT_6 receptor blockade decreases inhibitory synaptic transmission onto DGCs, via their direct effects on inhibitory interneurons. This hypothesis was investigated using extracellular and intracellular *in vitro* electrophysiology recording techniques in acute hippocampal brain

slices prepared from rats, as well as immunohistochemistry (IHC) in transgenic mice expressing an enhanced green fluorescent protein (EGFP) reporter upstream of the 5-HT_6 receptor gene (htr6-EGFP mice). Bath application of the 5-HT_6 receptor agonist WAY-208466 (1μM) attenuated fEPSP amplitude during extracellular recordings from perforant path-dentate granule cell (DGC) synapses and increased the amplitude of evoked inhibitory postsynaptic currents (eIPSCs) during voltage-clamp recordings from DGCs. In contrast, bath application of the 5-HT_6 receptor antagonist SB-399885 (1μM) attenuated eIPSC amplitude onto DGCs without affecting the amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs), and also produced a late increase in sIPSC interevent interval (IEI) during the washout phase. Interestingly, IHC in htr6-EGFP mice revealed that the majority of hilar mossy cells (GluA2-immunoreactive (IR)) were also GFP-IR, whereas only ~1% of GABA-IR neurons and DGCs were also GFP-IR. Results in this study suggest that 5-HT_6 receptors bidirectionally modulate inhibitory synaptic transmission onto DGCs via their direct effects on mossy cells and are consistent with the idea that 5-HT_6 receptor antagonists produce their memory enhancing effects via disinhibition.

Introduction

Memory deficits affect patients with a number of neurological disorders including dementia, epilepsy, Down's syndrome, autism-spectrum disorder, and psychiatric disorders (Selkoe, 2001; Helmstaedter, 2002; Cirillo and Seidman, 2003; Vicari et al., 2005; Boucher et al., 2012; Skodzik et al., 2013; Pauls et al., 2014). Memory impairment poses a significant risk of injury to patients and others and is thus among the primary

causes for admission into residential nursing homes (Andel et al., 2007). It was recently estimated that delaying admission into a residential care facility for dementia patients in the U.S. by only 1 month would cut healthcare spending by $4 billion (Gold and Budson, 2008). New treatments for memory deficits are needed because currently available therapies are few in number, mechanistically homogenous, only marginally effective, and produce side effects (Raina et al., 2008; Nygaard, 2013). One approach for future therapy discovery is to first obtain a mechanistic understanding of existing treatments, including those in development, in order to "fine-tune" their targeted effects or discover new therapeutic approaches.

One such promising treatment currently in development, serotonin (5-HT) 5-HT_6 receptor antagonists, have been shown to improve spatial and nonspatial memory in naïve, aged, and amnestic rodents during a variety of cognitive tasks including the Morris water maze, novel object recognition, social recognition, conditioned fear learning, and others (Rogers and Hagan, 2001; Woolley et al., 2001; Stean et al., 2002; Foley et al., 2004; King et al., 2004; Hirst et al., 2006; Mitchell et al., 2006; Da Silva Costa et al., 2009; Kendall et al., 2011) (see Fone, 2008 for a comprehensive review). On the strength of the preclinical data, 5-HT_6 receptor antagonists advanced to clinical trails in patients with mild-moderate AD and were shown to improve cognitive function in dementia patients; importantly, there were minimal adverse events (Maher-Edwards et al., 2010; Maher-Edwards et al., 2011; Wilkinson et al., 2014). However, gaps in our knowledge of 5-HT_6 receptor localization and function preclude a mechanistic understanding of their antagonists' therapeutic effects and the possibility of discovering new approaches to ameliorate cognitive dysfunction. Thus, the goal of this study was to dissect 5-HT_6

receptor localization and physiology with unprecedented precision in the DG.

Despite a number of studies reporting the nootropic effects of 5-HT_6 receptors, their cellular expression patterns and effects on synaptic transmission are not well understood. 5-HT_6 receptors are G-protein coupled receptors (GPCR) that positively couple to adenylyl cyclase, and their activity is linked to neuronal excitability (Millan et al., 2008). Accordingly, studies suggest 5-HT_6 receptor antagonists produce their memory enhancing effects by directly reducing the activity of inhibitory interneurons to facilitate information flow through excitatory networks – a phenomenon known as disinhibition (See Figure 2.1 for a schematic) (Dawson et al., 2001; Fone, 2008; Schechter et al., 2008; West et al., 2009; Codony et al., 2011). Support for the disinhibition hypothesis is derived from *in vivo* microdialysis studies in rats reporting that systemic administration of a 5-HT_6 receptor agonist increased extracellular levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) whereas a 5-HT_6 receptor antagonist increased extracellular levels of glutamate and acetylcholine in limbic brain areas expressing 5-HT_6 receptors (Dawson et al., 2001; Riemer et al., 2003; Lacroix et al., 2004; Schechter et al., 2008). West et al. (2009) reported that 5-HT_6 receptor activation increased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) onto pyramidal CA1 neurons with excitatory synaptic transmission pharmacologically blocked, implying that 5-HT_6 receptor activation directly increased the excitability of inhibitory synaptic transmission (West et al., 2009). Adding further support to the 5-HT_6 receptor antagonist-mediated disinhibition hypothesis, 5-HT_6 receptor mRNA and protein have been colocalized with striatal GABAergic neurons (Ward and Dorsa, 1996; Marazziti et al., 2012), and chemical ablation of serotonergic or cholinergic fibers failed

to alter 5-HT_6 mRNA expression, ruling out their role as autoreceptors (Gerard et al., 1996; Marcos et al., 2006).

Although the studies described previously are consistent with the hypothesis that 5-HT_6 receptors directly regulate the activity of inhibitory interneurons, this idea is not without controversy. For instance, 1 study reported 5-HT_6 receptor mRNA expression in pyramidal neurons, granule cells, and only a small subset of interneurons within the hippocampus (Helboe et al., 2015), and Wang et al. (2016) reported 5-HT_6 receptor agonist-mediated attenuation of glutamate release from hippocampal synaptosomes prepared from whole hippocampus homogenates (Wang et al., 2016), implying their expression on presynatic termini of excitatory hippocampal neurons. Moreover, representative examples of 5-HT_6 receptor expression on pyramidal neurons, interneurons, and astrocytes within the hippocampus have also been published (Woolley et al., 2004; Marazziti et al., 2013). However, confirmation and quantification of the cell-type specific expression patterns of 5-HT_6 receptors expression in the hippocampus remains unpublished, and is likely due to a lack of commercially available specific antibodies that are suitably well characterized. Moreover, the effects of 5-HT_6 receptor effects on synaptic transmission in the hippocampus, a brain region critical for memory formation (Squire, 1992), has been minimally reported, and their effects on synaptic transmission in the dentate gyrus (DG), the synaptic entranceway into the hippocampus, is lacking altogether.

Thus, the first aim of this study was to test the effects of 5-HT_6 receptor ligands on inhibitory synaptic transmission using *in vitro* electrophysiology in the DG of acute hippocampal brain slices. Preliminary data from these studies helped secure funding

generously granted from the Center on Aging at the University of Utah, which was allocated towards the second aim of this study: To determine cell-type specific expression patterns of 5-HT_6 receptors in the DG we obtained commercially available transgenic mice expressing a modified bacterial artificial chromosome (BAC) with an enhanced green fluorescent protein (EGFP) reporter upstream of the 5-HT_6 receptor gene (htr6-EGFP mice) (Gong et al., 2003; GENSAT, 2016).

We focused our investigation on the DG for several reasons. First, 5-HT_6 ligands have been shown to improve acquisition of spatial memory in rodents (Stean et al., 2002; Foley et al., 2004; Hirst et al., 2006), a function thought to be mediated by DG function (Lee et al., 2005; Goodrich-Hunsaker et al., 2008). Second, the primary output cells of the DG, dentate granule cells (DGCs), are heavily inhibited by local inhibitory interneurons that gate information flow into the hippocampus (Hsu, 2007), and thus may be an ideal anatomical location for 5-HT_6 receptor-mediated disinhibition. Lastly, preliminary data generated in our lab showed 5-HT_6 receptor activation attenuated basal excitatory synaptic transmission at perforant path-DGC synapses, a result that is consistent with the idea that 5-HT_6 receptor activation increased inhibitory synaptic transmission in the DG. Therefore, we hypothesized that 5-HT_6 receptor activation increases inhibitory synaptic transmission onto DGCs, whereas 5-HT_6 receptor blockade decreases inhibitory synaptic transmission onto DGCs, via their direct effects on inhibitory interneurons. Surprisingly, our results suggest that 5-HT_6 receptors indeed affect inhibitory synaptic transmission onto DGCs in a manner consistent with the disinhibition hypothesis – albeit indirectly, via their expression on mossy cells, an interesting class of local excitatory neuron in the hilus of the DG that drive inhibition

onto DGCs.

Methods

Animals

Electrophysiology experiments were conducted in hippocampal brain slices prepared from either Sprague Dawley male rats (100-200 g, Charles River, Raleigh, NC, U.S.A.). or C57BL/6 male mice (20.0 g, Charles River, Raleigh, NC, U.S.A.). Electrophysiology experiments conducted in mouse are clearly indicated in the text. Immunofluorescence studies were conducted in commercially available male and female htr6-EGFP mice on an FVB/N-Swiss Webster background. Htr6-EGFP mice express a modified bacterial artificial chromosome (BAC) containing an EGFP reporter upstream of the 5-HT_6 receptor gene (htr6). These mice were purchased from the Mutant Mouse Regional Resource Center at Jackson Laboratory (Gong et al., 2003; GENSAT, 2016). All animals were group housed in a light- and temperature-controlled (12 h on/12 h off) environment and permitted access to food and water *ad libitum* throughout the study. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Utah Institutional Animal Care and Use Committee. All efforts were made to minimize the number and suffering of animals used.

Hippocampal Brain Slice Preparation

Acute hippocampal brain slices were prepared daily from Sprague-Dawley rats (100-200 g) or a C57BL/6 mouse as done previously (West et al., 2014). Briefly, rats

were anesthetized with sodium pentobarbital (60 mg/kg, intraperitoneal (i.p.)), and brains were rapidly removed and submerged in ice-cold (4°C) oxygenated sucrose-based artificial cerebral spinal fluid (ACSF) bubbled with 95% O_2 / 5% CO_2. Sucrose-based ACSF contained the following (in mM): Sucrose (200.0), KCl (3.0), Na_2PO_4 (1.4), $MgSO_4$ (3.0), $NaHCO_3$ (26.0), glucose (10.0), and $CaCl_2$ (0.5). The pH and osmolarity of the sucrose-based ACSF were adjusted to 7.30-7.35 and 290–300 mOsm, respectively, before each experiment. Next, coronal brain slices (350μm) containing dorsal hippocampus were cut using a vibrating microtome (VT1000S, Leica Microsystems Inc.). Slices were then transferred to oxygenated standard ACSF and incubated for 2 h at room temperature prior to recording. Standard ACSF was made from the same recipe as sucrose-based ACSF, only sucrose was replaced with NaCl (126.0 mM), the $MgSO_4$ concentration lowered to 1.0 mM, and the $CaCl_2$ concentration raised to 2.5 mM.

Recording fEPSPs in the DG

Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded by transferring 8 coronal brain slices containing dorsal hippocampus from either hemisphere into the integrated brain slice chambers (IBSCs, Slicemates) of the Scientifica Slicemaster (Scientifica, Ukfield, East Sussex, U.K.), a high throughput semiautomatic brain slice recording system, which conducts separate but simultaneous extracellular recordings (Stopps et al., 2004). All slices were perfused with regular ACSF (2 mL / min) at 30°C unless noted otherwise. Homemade twisted Nichrome-Formvar stimulating electrodes were placed in the middle-third of the molecular layer in the upper blade of the DG to stimulate the medial perforant path; recording electrodes were placed in the distal

third approximately 400 – 600 μm away from the stimulating electrode. Input/output (I/O) relationships were measured by incrementally stimulating for 100 μs in 0.5 V steps until a population spike was observed or 20 V maximum stimulus intensity was reached. Data were acquired using pClamp 10 interfaced to a Digidata 1550A data acquisition board (Molecular Devices, Sunnyvale, CA, U.S.A.) at a sampling rate of 10 kHz, low-pass filtered at 1 kHz, and high-pass filtered at 3 Hz. The magnitude of the fEPSP was determined by measuring the peak amplitude in real-time.

After obtaining fEPSPs and conducting I/O curves, baseline stimulation strength was set to 50% and slices received 1 stimulation pulse every 30 s. The effects of the 5-HT_6 receptor agonist WAY-208466 on basal excitatory synaptic transmission along the perforant path-DGC synapse were studied in an experimental paradigm that took place in 3 20-min phases: Baseline, drug, and washout. During the baseline phase, we obtained stable fEPSPs with single stimulation pulses every 30 s for 20 min (fEPSP amplitudes that drifted > 20% from baseline stimulation strength were excluded). Next, slices were bath perfused with 100 nM, 300 nM or 1 μM of the 5-HT_6 receptor agonist WAY-208466, or ACSF (control slices) from a secondary reservoir for a 20 min drug phase, followed by a return to ACSF for a 20 min washout phase. Following the completion of experiments, the amplitudes of the fEPSPs were measured using pClamp software. fEPSP amplitudes were normalized by dividing their amplitude by the average amplitude of the last 4 fEPSPs obtained during the baseline phase and multiplied by 100. Normalized fEPSP amplitudes were then averaged for each concentration tested and expressed as an average percent of baseline ± the standard error of the mean (SEM).

Voltage-Clamp Recordings of eIPSCs, sIPSCs, and mIPSCs

To evaluate the effects of 5-HT_6 receptor activity on inhibitory synaptic transmission onto DGCs, the whole cell patch clamp technique was used for voltage clamp recordings. An individual brain slice from dorsal hippocampus was transferred to a perfusion chamber (RC-27L, Warner Instruments, Hamden, CT, U.S.A.), held between 2 nylon nets, and perfused with oxygenated ACSF (2 mL / min) at room temperature. During experiments testing the effects of the 5-HT_6 receptor agonist WAY-208466, patch electrodes (2.5-3.5 MΩ) were filled with the following internal solution (in mM): K-gluconate (140), HEPES (10.0), EGTA (1.0), $CaCl_2$ (0.5), and glucose (10.0), with the osmolarity set between 290–295 mOsm and the pH adjusted to 7.28 with KOH. All cells recorded from in the presence of a K^+-based internal were held at -70 mV. During experiments testing the effects of the 5-HT_6 receptor antagonist SB-399885, we wanted to optimize the signal-to-noise ratio for detecting spontaneous and miniature inhibitory postsynaptic events. Therefore, patch electrodes (2.5-3.5 MΩ) were filled with Cesium-based internal solution containing the following (in mM): Cs Methanesulfonate (CH_3CsO_3S, 140.0), HEPES (10.0), EGTA (1.0), $CaCl_2$ (0.5), Glucose (10.0), ATP (2.0), GTP (0.5), and QX-314 (5.0)–300 mOsm, pH=7.30 (CsOH). Cells recorded from in the presence of a Cs-based internal were held at 0 mV. All recordings were performed using a Multiclamp 700 B amplifier and the pClamp 10 (Clampex) software package interfaced to a Digidata 1440 A digitizer (Molecular Devices); recordings were digitized at 10 kHz and filtered at 1 kHz.

DGCs were “blindly” patched by forming tight seals (4-8 GΩ) with cells located in the granule cell layer of the DG with regular ACSF (2 mL / min) perfused at room

temperature. Once a granule cell was patched, all slices were then perfused with ACSF at 30°C. The effects of 5-HT_6 receptor agonist WAY-208466 and the 5-HT_6 receptor antagonist SB-399885 on evoked, spontaneous, and miniature inhibitory postsynaptic currents (eIPSCs, sIPSCs, and mIPSCs) took place in 3 separate experiments. First, we tested the effects of WAY-208466 on eIPSCs only. During this experiment a concentric bipolar stimulating electrode (MCE-100, Rhodes medical instruments, Summerland, CA, USA) connected to a stimulator was placed in the hilus of the DG (see Figure 2.2 panel A for schematic), and a paired-pulse stimulus with a 50 ms interpulse interval was given once every 30 s. In the second experiment, the effects of SB-399885 were tested using a similar paradigm, except sIPSCs were recorded during the 30-s sweeps, and stimulation of the hilus was performed with a Nichrome-Formvar stimulating electrode hereinafter. To include an ACSF control, some slices were bath perfused with regular ACSF coming from a secondary reservoir normally holding drug for 20 min, followed by a 10 min recovery to reestablish the eIPSC baseline; then slices were bath perfused with SB-399885. In the third experiment, we tested the effects of SB-399885 on mIPSCs with tetrodotoxin (TTX, 1 μM) present in the bath at all times to block sodium channel-mediated action potentials. mIPSCs were recorded continuously in 30-s sweeps.

All voltage-clamp experiments took place in 3 phases: Baseline, drug, and washout. Baseline phases were always 10 min in duration and drug phases always 20 min. The duration of the washout phase varied depending on whether recordings were unintentionally cut short, but was always at least 10 min in duration. Access and membrane resistance, holding current, and temperature were monitored in 30-s intervals using pClamp 10. Recordings accompanied by changes in access resistance > 25% that

exceeded 30 MΩ were excluded. Any cell exceeding 40 MΩ throughout the recording, regardless of its percentage change, was excluded**.**

Analysis of Voltage Clamp Data

All data analysis was completed offline following completion of each experiment. The amplitudes of eIPSCs were measured using pClamp 10 and were defined as the peak current injection between the rising and falling phase for each response to the paired-pulse stimulation (referred to as eIPSC 1 and 2). The average holding current 0.5 s prior the eIPSC was subtracted from this amplitude to give the true eIPSC amplitude. Paired-pulse ratio (PPR) was quantified as a percentage by dividing the amplitude of eIPSC2 by the amplitude of eIPSC1, multiplied by 100, and expressed as an average over time. The decay kinetics of eIPSC1 were measured by averaging the last 4 traces in the baseline, drug, and washout phases and fitting a single exponential decay curve to the falling phase.

The amplitude, interevent interval (IEI), length, and time constants for sIPSCs and mIPSCs were analyzed using Mini Analysis Program v6.0.1 (Synaptosoft, Fort Lee, NJ, U.S.A.). To limit inclusion of false positive events, the amplitude threshold for events was typically set to 10 pA (or a minimum of 2x the baseline noise) and event detection was confirmed visually. All amplitudes, IEIs, length, and time constants of sIPSCs and mIPSCs were measured and averaged over the first 20 s of each 30-s sweep. All eIPSC, sIPSC, mIPSC measurements (except PPRs) were normalized by dividing each measurement by the average of the last 4 events obtained during the baseline phase, multiplied by 100, and expressed as a percentage of the baseline average for each cell.

Following normalization, each parameter was then averaged for all cells recorded during that experiment, and expressed as a percentage ± SEM.

Immunohistochemistry

Sectioning, staining, and imaging of htr6-EGFP mice was conducted by Mr. Raunauk Basu (a graduate student in Dr. Megan Williams' lab). All cell counting and quantification was conducted by the author of this manuscript. Three htr6-EGFP mice and one nontransgenic control mouse (2 females and 2 males) between 3- and 6-months of age were transcardially perfused with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS) and the brains were carefully removed and soaked overnight in 4% PFA. Subsequently the brain was cut along the midline; one half was sectioned coronally, and the other horizontally into 100 μm sections. Sections were then incubated in blocking buffer (PBS with 3% BSA and 0.3% tritonX-100) for 1 h at room temperature and transferred to a Fab blocking solution (PBS with 3%BSA, 0.3% TritonX-100, and 2% Goat F(ab) anti-mouse IgG). Next, the sections were transferred to primary antibody solution comprising 1:3000 dilution of goat anti-green fluorescent protein (GFP) (Abcam, Cambridge, MA, U.S.A.), 1:2000 dilution of rabbit anti-GABA (Sigma-Aldrich, St. Louis, MO, USA), and 1:500 dilution of mouse anti-GluA2 (Millipore, Billerica, MA, U.S.A.) diluted in antibody dilution solution (PBS with 3% BSA and 0.2% Triton X-100) and incubated overnight at 4°C. Following incubation the sections were subjected to 3 washes in PBS (5 min per wash) followed by 1 h incubation (at room temperature) in secondary antibody mix comprising 1:1000 dilution of donkey anti-goat-Alexa488, donkey anti-rabbit-Cy5, and donkey anti-mouse-Cy3 (all from Jackson ImmunoResearch

Laboratories, West Grove, PA, U.S.A.) diluted in antibody dilution solution. The sections were washed 3 times with PBS and incubated in 1:10000 dilution of Hoechst for 5 min, placed on glass slides, and mounted for imaging in Fluoromount-G (Southern Biotech, Birmingham, AL, U.S.A.). The sections were imaged using a Zeiss LSM 710 confocal microscope.

Image Analysis

Quantification of cells bodies immunoreactive (IR) for GFP, GABA, or GluA2 in the DG were manually counted and recorded using the cell counter macro in ImageJ software (National Institutes of Health, Bethesda, MD). A cell was counted as GFP-IR or GABA-IR if its cell body was surrounded and at least partially or completely IR, whereas a cell was considered GluA2-IR if its soma surrounding the nucleus was IR. A cell was counted as a mossy cell if it was GluA2-IR (Leranth et al., 1996; Fujise and Kosaka, 1999), non-IR for GABA, had a neuronal somata that contained a visible nucleus (visualized with Hoescht staining) located within the hilus that was not in contact with the granule cell layer, and was outside the somal and dendritic regions of area CA3c. The DGC layer was defined as the upper and lower blade of densely packed neuronal somata between the hilus and the molecular layer, which was defined by a dense band of GFP staining immediately adjacent to the granule cell layer. All GFP-, GABA-, and GluA2-IR cells and their overlap were manually counted, quantified according to DG subregion, and averaged in 9 coronal sections from 3 htr6-EGFP mice and 6 horizontal sections from 2 of these 3 htr6-EGFP mice (1 ventral block was lost during sectioning). GFP-IR cells were expressed either as an average percent of a particular cell type expressing GFP (i.e.,

mossy cells, inhibitory interneurons, DGCs), or as a percentage of total GFP expression. All mean values are expressed ± SEM.

In order to estimate the percent of granule cells that were GFP-IR, an area of the upper or lower blade of the granule cell layer was randomly selected and measured (μm^2) in 3 separate areas at least 10 μm apart along the z-axis, and all visible nuclei between the molecular layer and hilus that were GluA2-IR were counted. The areas were then divided by the number of granule cells in that region to obtain an average area per granule cell (μm^2), so that the total number of granule cells in 1 z-plane could be estimated based on the entire area of the granule cell layer. The total area of the granule cell layer was then measured at 3 points along the z-axis separated by at least 10 μm in the z-plane. Each measurement was then divided by the estimated area per granule cell to yield the estimated number of granule cells. The number of GFP-IR cells was then counted in each of these z-planes, and divided by the estimated number of granule cells to yield an estimated percent of GFP-IR granule cells per z-plane. Estimated percentages of GFP-IR DGCS were quantified in 12 coronal z plans from 5 slices and 12 horizontal z-planes from 4 slices, averaged, and expressed as the mean ± SEM.

Chemicals and Drugs

Unless indicated otherwise, all chemicals and drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of WAY-208466 (Tocris Bioscience, Minneapolis, MN, U.S.A.), TTX and SB-399855 (Tocris Bioscience, Minneapolis, MN, U.S.A.) were made in ddH_2O at ≥ 1000 fold their final concentrations and stored at -20°C. On the day of the experiments, individual aliquots were defrosted and dissolved in

ACSF to the concentration indicated in each figure.

Statistics

For statistical comparison of all electrophysiology data, the average raw values of the last 4 measurements (1.5 min) during the baseline were compared to the average of the last 4 raw values in the drug phase using a paired Student's *t* test. Normalized fEPSP, eIPSC, or sIPSC amplitudes, as well as IEI were compared across drug or washout conditions to an ACSF control or other drug concentrations (when available) using an unpaired Student's *t* test for voltage-clamp data or one-way (nonrepeated measures) ANOVA with Bonferroni's multiple comparisons for fEPSP data. The slopes of linear regression fits were compared by F-test. All data sets were tested for normality using Kolmogorov-Smirnov test. When $p < 0.05$, or a data set had too few values to be tested for normality, Mann-Whitney U test or Wilcoxon signed-rank test was employed for unpaired or paired data, respectively. If unequal variances were detected, comparisons were made using Welch's unequal variances t-test. All statistical analysis was preformed using GraphPad Prism V5.0c (GraphPad Software, San Diego, CA); $p < 0.05$ was considered significant. *, **, or *** indicate a *p*-value of < 0.05, < 0.01, and < 0.001, respectively, for comparisons conducted within a particular condition. †, ††, or ††† was used to indicate a *p*-value of < 0.05, < 0.01, and < 0.001, respectively, when comparisons were conducted across conditions.

Results

The 5-HT_6 Receptor Agonist WAY-208466 Attenuated fEPSP Amplitude at Perforant Path-DGC Synapses

First, we wanted to test whether activation of 5-HT_6 receptors affected basal synaptic transmission in the DG. We generated fEPSPs at perforant path-DGC synapses in acute hippocampal brain slices prepared from rats and bath perfused control ACSF, or ACSF containing 100 nM, 300 nM, or 1 μM WAY-208466 (Figure 2.2). Although the average of the last 4 raw fEPSP amplitudes during the drug phase (38.5−40 min) were not significantly attenuated compared to baseline for any concentration tested, raw fEPSP amplitudes were significantly attenuated during the washout phase for 1 μM WAY-208466 (see Figure 2.2D for normalized data; raw values, 1 μM: baseline: -0.89 ± 0.06 mV; washout: -0.81 ± 0.06 mV, $p < 0.05$, paired Student's *t* test; 300 μM: baseline: -0.82 ± 0.11 mV; washout: -0.76 ± 0.11 mV, $p < 0.05$; paired Student's *t* test). There were also no significant differences in the averaged normalized fEPSP amplitudes across conditions during the last 2 min of the drug phase ($p > 0.05$, one-way ANOVA). However, there was a significant difference during the washout ($F_{(3, 39)} = 5.50$, $p < 0.01$, one-way ANOVA). Bonferroni's multiple comparisons revealed that 1 μM WAY-208466 significantly attenuated the normalized fEPSP amplitude during the washout phase compared to ACSF (Figure 2.2E, ACSF: 99.7% ± 1.1; 1 μM: 90.0 ± 2.6, $p < 0.01$). A continued effect of 5-HT_6 receptor activity on synaptic transmission during the washout phase has been reported in other studies testing the physiological effects of 5-HT_6 receptors *in vitro* (West et al., 2009) and is consistent with expectations when pharmacologically manipulating a GPCR. These results suggest that 5-HT_6 receptor activation attenuated

basal excitatory synaptic transmission at perforant path-DGC synapses and has lasting effects on synaptic transmission beyond the "washout" phase.

The 5-HT_6 Receptor Agonist WAY-208466 Increased the Amplitude of eIPSCs onto DGCs

Next we tested whether activation of 5-HT_6 receptors affects inhibitory synaptic transmission onto DGCs. We used voltage-clamp electrophysiology in acute hippocampal brain slices prepared from rats to record eIPSCs in DGCs by applying a paired-pulse stimulus once every 30 s to the hilus of the DG (see Figure 2.3A for experimental schematic). Bath perfusion of 1 μM WAY-208466 significantly increased the amplitudes of eIPSCs in the drug phase compared to baseline (Figure 2.3B-D, Baseline: 91.4 pA ± 30.3 pA; Drug: 122.7 ± 41.7 pA, $n = 7$, $p < 0.05$, Wilcoxon signed-rank test). We did not observe a change in the paired-pulse ratio (PPR) (Figure 2.3E, $p > 0.05$, Wilcoxon signed-rank test). Together, these results suggest that activation of 5-HT_6 receptors increased evoked inhibitory synaptic transmission onto DGCs without affecting presynaptic vesicular neurotransmitter release probability.

In a separate set of experiments performed 7 months after testing the effects of WAY-208466 on eIPSCs, 1 μM WAY-208466 no longer increased the amplitudes of eIPSCs onto DGCs (Figure 2.4, $n = 5$, $p > 0.05$, Wilcoxon signed-rank test). The irreproducibility of the effects of WAY-208466 on eIPSCs during this particular experiment could have been due to a number of experimental and / or biological phenomenon that are addressed in the discussion section. Importantly, the effects of WAY on eIPSC amplitude were reproduced at a later date in mouse. Nevertheless, it was

subsequently reasoned that if activation of the 5-HT_6 receptor no longer increased the amplitude of eIPSCs, perhaps blockade of the 5-HT_6 receptor with an antagonist would attenuate the amplitude of eIPSCs.

The 5-HT_6 Receptor Antagonist SB-399885 Attenuated the Amplitude of eIPSCs onto DGCs

Next, we tested the effects of the 5-HT_6 receptor antagonist SB-399885 on eIPSCs onto DGCs (Figure 2.5). Bath perfusing ACSF from a drug reservoir produced no significant effects on the amplitude of eIPSCs (Figure 2.5C, $n = 10$, $p > 0.05$, paired-students t-test). However, raw eIPSC amplitude was significantly attenuated in slices when SB-399885 was bath perfused (Figure 2.5C, D, eIPSC amplitude, Baseline: 391.7 ± 82.2 pA; Drug: 297.2 ± 71.7 pA, $n = 13$, $p < 0.01$, Wilcoxon signed-rank test). Additionally, SB-399885 significantly attenuated the normalized eIPSC amplitude during the drug phase compared to ACSF-treated slices (Figure 2.5C, ACSF: 112.8% ± 8.4; SB-399885: 73.1% ± 4.7, $p < 0.001$, unpaired Student's *t* test). We also measured eIPSC decay kinetics and observed no significant change in response in SB-399885 (Figure 2.5E, tau, Baseline: 30.6 ± 3.6 ms; Drug: 31.3 ± 3.9 ms, $p > 0.05$, $n = 12$ (unable to obtain an accurate measurement of decay kinetics for 1 cell), paired Student's *t* test). Similarly to WAY-208466, SB-399885 produced no significant change in the PPR (Figure 2.5F, $p > 0.05$, paired Student's *t* test). These results suggest that SB-399885 did not affect the open-time kinetics of GABA receptors on DGCs or the probability of presynaptic vesicle release at inhibitory synapses.

SB-399885 also did not significantly alter membrane resistances (data not shown,

n = 13, Baseline: 421.5 ± 110.6 MΩ; Drug: 554.8 ± 194.1 MΩ, $p > 0.05$, paired Student's t test), and the normalized membrane resistance values from SB-399885-treated slices did not significantly differ from ACSF during the drug phase (data not shown, ACSF: 114.1% ± 4.6; SB: 108.7% ± 11.8, $p > 0.05$, unpaired Student's t test). Access resistance did significantly increase during the drug phase in SB-399885 treated slices (Baseline: 18.79 ± 2.2 MΩ; Drug: 22.8 ± 2.7 MΩ, $p > 0.05$, paired Student's t test). However, the normalized values did not significantly differ from the ACSF control (ACSF: 111.5% ± 7.4; SB: 124.7% ± 11.4, unpaired Student's t test). Importantly, the increase in access resistance was not associated with an increase eIPSC decay kinetics (tau), which suggests changes in access resistance did not play a role in the attenuation in eIPSC amplitude observed in this study.

In summary, 5-HT_6 receptor blockade significantly attenuated evoked inhibitory synaptic transmission onto DGCs, which is the opposite effect of 5-HT_6 receptor activation illustrated in Figure 2.3. Moreover, we observed no significant changes in the PPR, tau decay kinetics of eIPSCs, or membrane resistance as a result of SB-399885. Thus, despite the irreproducibility of WAY-208466, results obtained insofar support the hypothesis that 5-HT_6 receptors bidirectionally control evoked inhibitory synaptic transmission onto DGCs in a manner consistent with 5-HT_6 receptor antagonist-mediated disinhibition via their presynaptic effects on interneuron excitability, as opposed to a postsynaptic effect on DGCs.

SB-399885 Produced a Delayed and Transient Increase in the IEI of sIPSCs

During experiments testing the effects of the SB-399885 on eIPSCs, we also recorded sIPSCs. Following experimentation, their average amplitude and IEI (the inverse of frequency) in ACSF- and SB-399885-treated slices were quantified (Figure 2.6, n = 10 and 13, respectively). We observed no differences in the amplitudes or IEIs of sIPSC when comparing the normalized values of drug and ACSF-treated slices across the drug phase (Figure 2.6B, C, $p > 0.05$, unpaired Student's t test), and no differences in the raw values between the baseline and drug phases for ACSF- or SB-399885-treated slices (Figure 2.6D, E, $p > 0.05$, paired Student's t test for amplitude, $p > 0.05$, Wilcoxon signed-rank test for IEI). However, we did observe a late effect in IEI, as the normalized mean sIPSC IEI was increased compared to ACSF-treated slices during the washout phase (Figure 2.6C, ACSF: 103.4% ± 5.2; SB-399885: 141.8% ± 11.7, $p < 0.05$, Welch's unequal variances t test) and the raw sIPSCs IEI during the washout phase were significantly increased compared to baseline in SB-399885-treated slices (Figure 2.6E, Baseline: 1,274.0 ± 455.0 ms; Washout: 1,725.0 ± 514.3 ms, n = 13, $p < 0.01$, Wilcoxon signed-rank test). Thus, the number and amplitude of sIPSCs onto DGCs was unaffected by the 5-HT_6 receptor antagonist until the washout phase, during which we observed a decrease in the average number of spontaneous inhibitory postsynaptic events in DGCs. Although the reason why this late effect occurs with a large delay is not presently known, the increase in the IEI (i.e., a decrease in the frequency) of sIPSCs is consistent with the hypothesis that 5-HT_6 receptor blockade attenuated inhibitory synaptic transmission.

Does SB-399885 Affect the Amplitudes of mIPSCs onto DGCs?

To determine whether 5-HT_6 receptor blockade affects presynaptic inhibitory neurotransmitter vesicle release onto DGCs and has postsynaptic effects in DGCs, we tested the effects of the 5-HT_6 receptor antagonist SB-399885 on mIPSCs in the presence of 1 μM TTX (Figure 2.7). SB-399885 significantly attenuated the average amplitude of mIPSCs (Figure 2.7D, Baseline: 19.8 ± 0.8 pA; Drug 17.2 ± 0.6 pA, $n = 13$, $p < 0.01$, paired Student's t test), but had no significant affect on mIPSC IEI (Figure 2.7E, $n = 13$, $p > 0.05$; paired Student's t test), suggesting that 5-HT_6 receptors had postsynaptic effects on DGCs. However, linear regressions fit to the baseline, drug, and washout phases of the normalized mIPSC amplitudes (0−10, 10−30, 30−40 min, respectively) revealed that the slope of all 3 lines are significantly different from 0 (Figure 2.7B, Slope, Baseline: -0.22 ± 0.10, $p < 0.05$; Drug: -0.15 ± 0.03 $p < 0.0001$; Washout: -0.10 ± 0.01, $p < 0.0001$, linear regression) and are not significantly different from each other ($p > 0.05$). For comparison, linear regressions fit to an identical time course of eIPSC amplitudes in SB-399885 treated slices (Figure 2.5B) revealed that the slope in the baseline and washout phases do not significantly differ from 0, whereas the slope for the drug phase significantly differs from 0 (Slope, Baseline: -0.35 ± 0.33, $p > 0.05$; Drug: -1.52 ± 0.12, $p < 0.0001$; Washout: -0.40 ± 0.39, $p > 0.05$, linear regression). Moreover, these slopes are significantly different from each other ($p < 0.001$). Thus, linear regression analysis suggests that mIPSC amplitudes were decreasing during the baseline phase, and the rate of change was not significantly altered by bath application of SB-399885, whereas eIPSC amplitudes were flat until SB-399885 produced a significant decrease in eIPSC amplitude. Together, these results suggest that SB-399885 *did not* attenuate the

amplitudes of mIPSCs via postsynaptic effects in DGCs and *did not* affect the probability of presynaptic vesicle release in inhibitory presynaptic termini synapsing onto DGCs.

GFP Immunoreactivity in htr6-EGFP Mice Is Primarily Found in Excitatory Neurons

During this time, the aforementioned results were used as preliminary data to obtain seed funding generously granted to Dr. Peter J. West from the Center on Aging at the University of Utah. These funds were used to purchase BAC transgenic mice expressing an EGFP reporter under the 5-HT_6 receptor promoter (Gong et al., 2003; GENSAT, 2016). Immunofluorescence staining was used to quantify GFP-immunoreactive (IR) cell types based on colocalization with γ-aminobutyric acid (GABA), a marker for inhibitory interneurons, GluA2, a marker that is specific to mossy cells in the hilus of the DG (Leranth et al., 1996; Fujise and Kosaka, 1999), and their neuroanatomical localization within DG subregion: the DGC layer, the molecular layer, or the hilus. Representative images of staining in horizontal and coronal sections can be found in Figures 2.8 and 2.9, respectively. A summary of the quantification can be found in Table 2.1.

Immunofluorescence experiments in tissue from htr6-EGFP mice revealed an interesting and unexpected staining pattern in the DG. 81.1% ± 4.1 of mossy cells in horizontal slices (containing primarily ventral hippocampus) were GFP-IR, whereas 66.2% ± 4.4 of mossy cells in coronal slices (containing primarily dorsal hippocampus) were GFP-IR. Surprisingly, only 0.3% ± 0.1 of GABA-IR cells in horizontal slices and 1.3% ± 0.4 of GABA-IR cells in coronal slices were also GFP-IR. This small population

of cells that were colabeled with GFP and GABA were always located in the hilus. Additionally, based on estimates of the total number of DGCs per slice, 1.2% ± 0.3 of DGCs in horizontal slices and 1.1% ± 0.1 of DGCs in coronal slices were GFP-IR. In summary, the majority of mossy cells were GFP-IR, and approximately 1% of DGCs and inhibitory interneurons were GFP-IR.

As a percentage of total GFP-IR cells observed in the entire DG, GFP-IR mossy cells accounted for 65.6% ± 11.6 in horizontal slices but only 26.2% ± 2.9 in coronal slices. GFP-IR DGCs accounted for 29.2% ± 10.0 of total GFP expression in the DG in horizontal slices and 40.0% ± 3.4 in coronal slices, whereas cells colabeled with GFP and GABA accounted for 0.3% ± 0.2 of total GFP expression in horizontal slices, and 0.7 ± 0.3% in coronal slices. Additionally, 3.0% ± 1.6 of GFP-IR cells in horizontal slices were located just outside the granule cell region in the molecular layer, whereas 15.1% ± 2.9 of EGFP-IR cells in coronal slices were located in the molecular layer. This population of GFP-IR cells found in the molecular layer never colocalized with GABA, and always colocalized with GluA2, which suggests they are excitatory neurons. Lastly, 1.9% ± 1.2 of GFP-IR cells in horizontal slices did not colocalize with GluA2 or GABA, but had visible nuclei revealed via Hoescht staining. Interestingly, this population of cells accounted for 18.0% ± 2.3 of EGFP-IR cells in coronal slices. Although additional experiments are needed to identify this population of cells, we suspect that these GFP-IR cells lacking GABA or GluA2 labeling may be astrocytes, a finding that would parallel other reports of 5-HT_6 receptors on astrocytes (Marazziti et al., 2013). Importantly, we also stained horizontal and coronal sections prepared from nontransgenic littermates of htr6-EGFP mice that do not express EGFP; we did not observed any GFP-IR cells in

these sections. See Figures 2.8A and 2.9A for representative images.

In summary, the majority of mossy cells in both coronal and horizontal cut sections were GFP-IR, whereas very little GFP-immunoreactivity (~1%) was observed in DGCs and GABA-IR cells. The majority of total GFP-immunoreactivity in the DG of horizontally cut slices was found in mossy cells, followed by DGCs, molecular layer cells, an unknown cell type that possibly represent glia, and lastly, GABA-IR cells, whereas the majority of total GFP-immunoreactivity in the DG of coronally cut slices was observed in DGCs (but only accounted for ~1% of the total population of DGCs), followed by mossy cells, an unknown cell type in the hilus that may be astrocytes, molecular layer cells, and lastly, GABA-IR cells. See Table 2.1 for a summary of these quantifications.

Preliminary Data: 5-HT_6 Receptor Ligands Have Similar Effects on Inhibitory Synaptic Transmission in Mice and Rats

Lastly, we also tested the effects of a 5-HT_6 receptor antagonist and agonist on eIPSCs onto DGCs in hippocampal brain slices prepared from mice (Figure 2.10, $n = 1$). Although this experiment was conducted only once and needs to be repeated, we found that bath application of 1 μM SB-399885, followed by a washout phase and bath application of 1 μM WAY-208466 resulted in an attenuation of eIPSC amplitude, followed by an increase in eIPSC amplitude. This finding is important because the effects on eIPSC amplitude closely parallel the effects of 5-HT_6 receptor ligands in hippocampal brain slices prepared from rats.

Discussion

This study investigated 5-HT_6 receptor localization and physiology in the DG using *in vitro* electrophysiology and IHC. Extracellular recordings of basal excitatory synaptic transmission revealed that the 5-HT_6 receptor agonist WAY-208466 dampened fEPSP amplitude at perforant path-DGC synapses. During voltage-clamp recordings from DGCs, WAY-208466 also increased the amplitudes of electrically evoked IPSCs onto DGCs, an effect that, for reasons unknown, was irreproducible 7 months later. However, the next experiment showed the 5-HT_6 receptor antagonist SB-399885 produced the opposite effect by attenuating the amplitude of eIPSCs, without affecting their decay kinetics, and also produced a late decrease in the number of sIPSCs onto DGCs without affecting their amplitudes. SB-399885 may have attenuated the amplitude of mIPSCs, however, linear regression analysis suggests this effect may have due to nonspecific rundown in the amplitudes of the mIPSCs (drift). Thus, 5-HT_6 receptor activity bidirectionally affected eIPSCs onto DGCs in a manner consistent with 5-HT_6 receptor antagonist-mediated disinhibition. Interestingly, quantitative immunohistochemical analysis in the DG of htr6-EGFP mice revealed that the majority of mossy cells, an excitatory interneuron type in the hilus, were immunoreactive for GFP, whereas only ~1% of DGCs and inhibitory interneurons were immunoreactive for GFP. The most parsimonious explanation of results obtained from electrophysiology and IHC experiments suggest that 5-HT_6 receptors bidirectionally and indirectly modulate inhibitory synaptic transmission onto DGCs via their direct effects on mossy cells (see Figure 2.11).

The novel hypothesis that 5-HT_6 receptor ligands bidirectionally control

inhibitory synaptic transmission onto DGCs via their direct effects on mossy cells comes primarily from results obtained in htr6-EGFP mice showing the majority of mossy cells were GFP-IR, the lack of effect of SB-399885 on sIPSC amplitudes despite a concurrent decrease in eIPSC amplitude, and lastly, their lack of effect on eIPSC decay kinetics. This idea is also supported by other studies reporting that mossy cells are excitatory neurons that drive inhibition onto DGCs. For example, using dual whole-cell patch clamp technique, Scharfman (1998) showed that action potentials produced in mossy cells produced a monosynaptic depolarizing event in DGCs when inhibition was pharmacologically blocked, but faithfully caused a di-synaptic inhibitory postsynaptic potential (IPSP) in DGCs with inhibition intact (Scharfman, 1995). Moreover, Jinde et al. (2012) showed that near-perfect selective ablation of mossy cells attenuated the frequency of both sIPSCs and sEPSCs in DGCs, but rendered DGCs hyperexcitable, as evidence by increased synaptic responsiveness, and reduced threshold to population spike. Together, these studies suggest that mossy cells innervate both DGCs and inhibitory interneurons, but the net effect of their activity drives inhibition onto DGCs. Although very little is known about how 5-HT_6 receptors regulate neuronal excitability, additional support for the idea that 5-HT_6 receptors could indirectly affect inhibitory synaptic transmission via their direct effects on mossy cells comes from a study by Bonsi et al. (2007) showing that blockade of 5-HT_6 receptors hyperpolarized cells expressing 5-HT_6 receptor mRNA in the striatum via K^+-dependent effects, (Bonsi et al., 2007), which suggests that 5-HT_6 receptors could regulate mossy cell excitability via their effects on leak potassium channels (Millan et al., 2008). Taken together, the results obtained in the present study, as well as those reported by others, suggests that pharmacological

activation of 5-HT_6 receptors with WAY-208466 directly increased the excitability of mossy cells and their excitatory drive onto inhibitory interneurons, and thereby increased the excitability of inhibitory interneurons that synapse onto DGCs, such that electrically evoked stimulation of the hilus recruited a greater number of inhibitory interneurons in the presence of WAY-208466 and thereby produced an eIPSC greater in magnitude. Likewise, the data presented here suggest that the 5-HT_6 receptor antagonist attenuated inhibitory synaptic transmission by directly reducing the excitability of mossy cells and thereby attenuating inhibition onto DGCs (see Figure 2.11 for a schematic).

Although this hypothesis is consistent with the majority of our findings and the aforementioned studies published by others, not all of our findings fit with this hypothesis and need to be addressed. First, if SB-399885 did decrease the amplitudes of eIPSCs by indirectly (or even directly) altering the excitability of inhibitory interneurons synapsing onto DGCs, we would expect the frequency of sIPSCs to change in a manner that is temporally and directionally coincident with changes in eIPSC amplitudes. We did observe a delayed decrease in the average number of sIPSCs during the washout phase (Figure 2.6), but the effect on sIPSCs did not occur simultaneously with the decrease in the amplitudes of eIPSCs. Moreover, the increase in the IEI of sIPSCs during the washout phase was not observed in all recordings. This may suggest that the decrease in eIPSC amplitudes were mediated by mechanisms other than a change to the excitability of inhibitory interneurons, such as a postsynaptic effect on DGCs. However, additional analysis revealed that the majority of our recorded events are not likely action potential-driven events and are comprised mostly of mIPSCs (analysis not shown). Thus, subtle changes in action potential-driven sIPSC frequency may have been indistinguishable in

the presence of events that were mostly comprised of mIPSCs. Moreover, a neuron's intrinsic excitability is not linearly related to the number of spontaneous action potentials it fires (Jiang et al., 2015). Thus, it is more likely that the amplitudes of eIPSCs decreased in the presence of SB-399885 as a result of a decrease in the excitability of inhibitory interneurons without immediately affecting the number of sIPSCs onto DGCs.

Another result that conflicts with the hypothesis that 5-HT_6 receptors orchestrated changes to inhibition onto DGCs via mossy cells is that 5-HT_6 receptor blockade attenuated the amplitudes of mIPSCs in a manner temporally coincident with the decrease in eIPSC amplitude (Figures 2.5 and 2.7). The canonical interpretation of this result suggests that 5-HT_6 receptors dampened mIPSC amplitudes by reducing the number and/or conductance of $GABA_A$ receptors ($GABA_A$Rs) directly in DGCs expressing 5-HT_6 receptors. Since DGCs were blind-patched (and thus, randomly selected) in hippocampal brain slices prepared from naïve rats, it is unlikely we faithfully recorded from the ~1% of DGCs that, according to our IHC data, express 5-HT_6 receptors. Thus, the most parsimonious explanation for the attenuation in mIPSC amplitudes is that 5-HT_6 receptors are expressed in the majority of DGCs. This result could explain some of the electrophysiology results obtained in the present study, including the effects of 5-HT_6 receptor ligands on eIPSCs and fEPSPs. 5-HT_6 receptor expression in DGCs would also fit with the results of Helboe et al. (2015) who reported 5-HT_6 receptor mRNA in >70% of DGCs in rats, as well as findings recently reported in an online public resource published by Janelia Research Campus (Cembrowski et al., 2016), where whole genome characterization of mRNA expression in mice shows minimal expression of 5-HT_6 receptor mRNA in mossy cells and high levels of expression in DGCs. One explanation

for these results is that mossy cells may transcribe minute amounts of 5-HT_6 mRNA into protein, whereas DGCs may not transcribe the high amounts of 5-HT_6 receptor mRNA into protein. If the majority of DGCs do express 5-HT_6 receptors, either endogenous 5-HT_6 receptor expression in rat differs from mouse (although preliminary data in Figure 2.10 suggests 5-HT_6 receptor ligands affect inhibitory synaptic transmission similarly in mouse and rat), or GFP immunoreactivity in BAC transgenic mice does not accurately reflect endogenous 5-HT_6 receptor expression in either species. However, linear regression analysis suggests that the amplitudes of mIPSCs were already decreasing during the baseline phase and SB-399885 did not influence the continued rate of decrease. Thus, mIPSC amplitudes may have been *unaffected* by SB-399885. Considering the entirety of the data that is inconsistent with a direct effect of 5-HT_6 ligands on DGCs, as well as the poor baseline control and likely downward drift observed in this particular data set, we conclude that the decreased mIPSC amplitudes observed here are based on unreliable data. Accordingly, we have excluded these data from our overall conclusions regarding our model of where 5-HT_6 receptors are expressed and their physiological roles on modulating inhibitory synaptic transmission in the DG.

Another result that can be explained by 5-HT_6 receptor expression in mossy cells or DGCs is that bath application of the 5-HT_6 receptor agonist WAY-208466 attenuated the amplitude of fEPSPs in hippocampal brain slices. Although this result is consistent with a postsynaptic effect of 5-HT_6 receptor on DGCs, the mechanics of this phenomena are complex and warrant further discussion. The fEPSP is primarily an excitatory postsynaptic response that is proportional to the magnitude of positive ions (i.e., Na^+) entering DGCs via glutamate receptors following perforant-path stimulation. Thus, the

canonical interpretation of this result is that the attenuation of fEPSP amplitude is due to a reduction in the conductance and / or number of glutamate receptors inserted in the postsynaptic membrane, suggesting that 5-HT_6 receptors located on DGCs mediated the WAY-208466-induced attenuation of fEPSP amplitude. However, another phenomenon known as shunting inhibition may have contributed to the attenuation of fEPSP amplitude. Shunting inhibition can be defined as the opening of extrasynaptic GABA receptors that produces a reduction in the local membrane resistance, and thus attenuated the amplitude of incoming excitatory postsynaptic potentials (Hao et al., 2009; Paulus and Rothwell, 2016). Support for the idea that shunting inhibition could have attenuated fEPSP amplitudes is derived from another study reporting that systemic administration of WAY-208466 increased the extracellular concentration of GABA in the hippocampus (Schechter et al., 2008). Although we did not observe a significant change in membrane resistance during patch clamp experiments, we did observe a nonsignificant increase in the membrane resistance of DGCs during experiments with SB-399885, an effect that would be expected if extracellular concentrations of GABA decreased.

Together, these results suggest that 5-HT_6 receptors bidirectionally modulate inhibitory synaptic transmission onto DGCs via their direct effects on mossy cells. It is, however, still possible that our results are explained by 5-HT_6 receptor expression on DGCs that alter GABA receptor responses. Moreover, these competing hypotheses need not be mutually exclusive; both presynaptic and postsynaptic mechanisms may have contributed to changes in inhibitory synaptic transmission. Additional experiments are needed to test their individual and perhaps combined contributions to 5-HT_6 receptor-mediated changes to inhibition in DGCs.

In order to begin testing whether mossy cells are physiologically responsive to changes in 5-HT_6 receptor activity, we attempted to record from GFP+ neurons in brain slices prepared from htr6-EGFP mice and naïve mice. Despite clear identification of GFP-IR cells in fixed tissue using IHC, we observed minimal and weak fluorescence in acute brain slices prepared from htr6-EGFP that prevented recording from labeled neurons. Weak fluorescence could have been due to photobleaching during the slicing and incubation process, as slices were made and incubated in normal lighting. Future attempts to patch clamp GFP+ neurons in acute brain slices prepared from htr6-EGFP mice should take greater precaution to protect the slices from photobleaching.

We also attempted to directly patch clamp mossy cells in brain slices prepared from naïve mice. Our efforts to patch mossy cells in our pilot study were hampered by the fragility of mossy cells and our inability to form a tight seal on them; unfortunately, we were unable to obtain any successful patch clamp recordings from mossy cells. Future experiments could improve our chances of recording from mossy cells by altering our brain slice preparation protocol to improve slice health (see www.brainslicemethods.com). Additionally, we could also use the "cleaning" technique, which employs suction via a glass capillary to remove dead tissue and debris on top of the brain slice that prevents visualizing cells buried deep in the slice. This could allow us to visually-patch mossy cells that are deep in the tissue and presumably in better health than those near the surface. Lastly, we could contact another lab (i.e., Dr. Helen Sharfman at New York University) that routinely record from mossy cells to obtain advice. Once we are able to record from mossy cells, a variety of current clamp protocols could be used to determine how 5-HT_6 receptor ligands affect mossy cell excitability.

One experiment that could begin to test our hypothesis that 5-HT_6 receptors modulate inhibitory synaptic transmission onto DGCs would be to retest the effects of a 5-HT_6 receptor agonist or antagonist on eIPSC amplitude in the presence of drugs that block excitatory synaptic transmission (i.e., CNQX and APV). If mossy cells directly mediate the effects of 5-HT_6 receptor ligands on inhibitory synaptic transmission, we would expect to see no change in eIPSC amplitude with excitatory synaptic transmission pharmacologically blocked. Conversely, a change in the amplitude of eIPSCs onto DGCs would rule out the role of mossy cells and suggest that 5-HT_6 receptor activity affects inhibitory synaptic transmission onto DGCs via their direct effects on DGCs and/or inhibitory interneurons expressing 5-HT_6 receptors.

Although the aforementioned experiments would begin to test our hypothesis, several other mechanisms could have mediated the observed effects of 5-HT_6 receptor ligands on inhibitory synaptic transmission onto DGCs. For instance, in addition to mossy fiber projections onto CA3 pyramidal neurons, DGCs make axon collaterals that innervate hilar inhibitory interneurons and mossy cells. Although ~1% of DGCs were GFP-IR (according to our IHC data), GFP-IR DGCs made up the largest percentage of total GFP staining in the DG in coronally cut sections (Table 2.1), and all electrophysiology experiments in this study were conducted in coronally cut sections. Therefore, it is possible that 5-HT_6 receptor ligands altered the intrinsic excitability of a subpopulation of DGCs, altering their excitatory drive onto inhibitory interneurons or mossy cells that innervate inhibitory interneurons and thereby affecting the amplitude of eIPSCs. In retrospect, we could have tested this hypothesis by conducting current clamp experiments in the DGCs we recorded from during these experiments. However, in most

of these experiments our internal solution ($CsMeSO_4$), which fills the neuron during whole-cell recording, was optimized for recording spontaneous events in a manner that blocks potassium channels and thus prevented the study of neuronal excitability. We did not, however, observe any significant changes in membrane resistance associated with bath application of SB-399885 during these experiments, which suggests 5-HT_6 receptor ligands did not affect the intrinsic excitability of DGCs via leak potassium channels. However, additional experiments are needed to comprehensively test whether 5-HT_6 receptors affect DGC intrinsic excitability.

Another possibility is that CA3 pyramidal neurons, the majority of which were found to be GFP-IR (data not shown), played a role in affecting inhibitory synaptic transmission onto DGCs. CA3c pyramidal neurons back-project to the DG and primarily make synapses onto hilar inhibitory interneurons and mossy cells (Scharfman, 2007). Thus, CA3 pyramidal neurons may have had altered excitatory drive onto these hilar neurons in the presence of 5-HT_6 receptor ligands and therefore contributed to the results observed in this study. Additionally, we also observed a population of GFP-IR cells that were almost exclusively found in the hilus of coronally cut sections and did not overlap with GluA2 or GABA-IR, but did have a nucleus. Although additional experiments are needed to further explore their identity, this population of GFP-IR cells may have been astrocytes. In support of this idea, Marazitti et al. (2013) showed a representative example of 5-HT_6 receptor antibody staining that overlapped with glial fibrillary acidic protein (GFAP), a marker for astrocytes, in human hippocampal tissue. Furthermore, astrocytes have been shown to release GABA and play a role in modulating inhibitory synaptic transmission (Kozlov et al., 2006; Wu et al., 2014), and therefore may have

played a role in 5-HT_6 receptor-mediated changes in inhibitory synaptic transmission. Thus, future experiments should confirm whether 5-HT_6 receptors are expressed on astrocytes and test their potential role in mediating inhibitory synaptic transmission in the DG.

In summary, the effects of 5-HT_6 receptor ligands on inhibitory synaptic transmission could have been mediated by a variety of cell types found to express GFP including mossy cells, DGCs, CA3 pyramidal neurons, and/or astrocytes. However, our results showing that 5-HT_6 receptor ligands bidirectionally control the amplitudes of evoked inhibitory synaptic transmission onto DGCs without affecting their decay kinetics or attenuating the amplitudes of sIPSCs, along with staining in tissue from htr6-EGFP mice illustrating that the majority of mossy cells were GFP-IR, suggest that 5-HT_6 receptors affect inhibitory synaptic transmission onto DGCs via mossy cells. Support for this hypothesis also comes from studies showing that mossy cells drive inhibitory synaptic transmission onto DGCs (Scharfman et al., 2003; Jinde et al., 2012), and that 5-HT_6 receptor activity can affect the resting membrane potential in neurons expressing 5-HT_6 receptor mRNA (Bonsi et al., 2007). Thus, we hypothesize that 5-HT_6 receptor activity regulates inhibitory synaptic transmission onto DGCs via their direct effects on mossy cells.

Although our results adequately support this hypothesis, there are a number of methodological limitations in the present study that need to be addressed. The first stems from the use of htr6-EGFP mice. Although BAC transgenic mice are a powerful tool for dissecting cell-type specific expression patterns, and gross anatomical staining of GFP expression in htr6-EGFP mice on the providers website (GENSAT, 2016) matches

regional expression patterns in mice shown by others using radioligand binding assays and *in-situ* hybridization (Hirst et al., 2003), validation that GFP expression in htr6-EGFP mice mirrors endogenous cell-type specific 5-HT_6 receptor expression is lacking, in part, due to a dearth of well characterized commercially available 5-HT_6 receptor antibodies. Thus, researchers need to exhibit caution when interpreting cell-type specific staining patterns in htr6-EGFP BAC transgenic mice.

Another limitation in the present study is that almost all electrophysiology recordings performed were conducted in hippocampal brain slices prepared from rats (except for Figure 2.10), whereas the IHC experiments were performed in transgenic mice, thus creating some concern regarding species differences. We conducted the electrophysiology portion of this research in brain slices prepared from rats because most behavioral studies testing the effects of 5-HT_6 receptor ligands on learning and memory have been conducted in rat (Fone, 2008). Additionally, other studies have reported species differences in 5-HT_6 receptor homology that reduce the affinity of 5-HT_6 receptor ligands in mice, and caution against conducting 5-HT_6 receptor studies in mice (Hirst et al., 2003; Setola and Roth, 2003). Although additional experiments are needed before definitive conclusions can be drawn, our data in Figure 2.10 (n = 1) suggests that 5-HT_6 receptors in mice are responsive to 5-HT_6 ligands and affect inhibitory synaptic transmission similarly in mouse and rat (data in Chapter 3 of this dissertation also suggest that 5-HT_6 receptor ligands affect behavior in mice as well). However, we must exhibit caution when drawing conclusions between data obtained in mouse and rats.

Another point worthy of discussion is the irreproducibility we observed when re-testing the effects of the 5-HT_6 receptor agonist WAY-208466 on eIPSCs. Bath

application of 1μM WAY-208466 consistently increased the amplitude of eIPSCs onto DGCs in 2013, but no longer had an effect 7 months later. Although the reason for this irreproducibility remains unknown, others have reported that 5-HT_6 receptors can exhibit high levels of constitutive activity when expressed in various cell lines (Kohen et al., 2001; Grychowska et al., 2016). Therefore, we hypothesized that 5-HT_6 receptors may have, for reasons currently unknown, become constitutively activated, thus preventing further activation. This hypothesis eventually led us to test the 5-HT_6 receptor antagonist SB-399885, which attenuated the amplitudes of eIPSCs. We also tested the effects of WAY-208466 in a similar experiment in a brain slice prepared from a mouse (Figure 2.10, n = 1). Importantly, the effects 5-HT_6 receptor ligands on eIPSC amplitude in mouse mirrored the results obtained rat. However, the observed irreproducibility of WAY-208466 in rat hippocampal brain slices remains a mystery.

Despite these limitations, to date, there are no published studies testing the effects of 5-HT_6 receptor activity on synaptic transmission in the DG, examining GFP staining in htr6-EGFP mice, or studies suggesting 5-HT_6 receptor expression on mossy cells. A number of publications hypothesize that 5-HT_6 receptor activity influences excitatory synaptic transmission via their direct effects on inhibitory interneurons (Dawson et al., 2001; Woolley et al., 2004; Fone, 2008; Schechter et al., 2008; West et al., 2009; Codony et al., 2011; Ramirez, 2013). However, other studies have reported 5-HT_6 receptor and mRNA expression on other cell types in the hippocampus such as DGCs, pyramidal neurons, and astrocytes (Woolley et al., 2004; Marazziti et al., 2013; Helboe et al., 2015). This controversy stems from, in part, a lack of 5-HT_6 receptor-specific antibodies and thus, reliance on mRNA expression to dissect the cell-type specific expression pattern of

5-HT_6 receptors. Although our results in htr6-EGFP need to be confirmed, they suggest that excitatory mossy cells are an entirely novel cellular mediator between 5-HT_6 receptors and their effects on inhibitory synaptic transmission.

Besides prominent GFP immunoreactivity in mossy cells, IHC in htr6-GFP mice yielded several interesting results with respect to GFP staining patterns in coronal versus horizontal sections. For example, although the number of htr6-EGFP mice examined was low, and the precision used during the slicing process was insufficient to delineate differences in dorsal-ventral hippocampus in this study, the percentage of GFP-IR mossy cells in horizontal sections (81%), which presumably contain ventral hippocampus, was greater than the percentage of GFP-IR mossy cells in coronal sections (66%), which presumably contain dorsal hippocampus. Furthermore, mossy cells accounted for the largest population of all GFP-IR cells in horizontal sections (~65%), whereas DGCs accounted for the largest population of all GFP-IR cells in coronal sections (~40%). Another notable difference between coronal and horizontal slices is the occurrence of GFP-IR cells that were not GluA2- or GABA-IR but contained a nucleus. This population, which may have been astrocytes, made up almost 20% of total GFP-IR cells in coronal sections, but only ~2% in horizontal sections. Lastly, we also observed a population of GFP-IR cells in the molecular layer that accounted for ~15% of the all EGFP-IR cells in coronal slices. Their cell bodies were often found on the border of the DGC layer and molecular layer, were never GABA-IR, and were always GluA2-IR. Interestingly, there is a population of excitatory neurons known as semilunar granule cells (SGCs) that reside on the DGC-molecular layer border. Very little is known about this recently discovered population of neurons, however, SGCs receive perforant-path

inputs and send axons to hilar and CA3 neurons, but differ from DGCs in that they have high intrinsic excitability and exhibit a sustained unaccommodating firing pattern upon depolarization (Williams et al., 2007). It is suspected that this population of GFP-IR neurons could be SGCs, however, additional studies are needed to confirm their identity. Future studies should confirm the interesting 5-HT_6 receptor expression patterns observed in the present study, perhaps using 5-HT_6 receptor antibodies that may be developed in the future, and determine how their expression patterns affect DG physiology and function.

In summary, our results are consistent with other studies suggesting that 5-HT_6 receptor antagonist may exert their nootropic effects via disinhibition. Augmenting neural circuits via disinhibition is a fundamental theme in nervous system function and may be targetable for memory enhancement and remediation of memory deficits in disease (Froemke, 2015; Letzkus et al., 2015). Additionally, several studies suggest that mossy cells play a role in mediating spatial pattern separation and contextual fear memory and thus, 5-HT_6 receptors may be ideally anatomically situated on mossy cells to mediate 5-HT_6 receptor antagonists' procognitive effects by disinhibiting DGCs (Myers and Scharfman, 2009; Jinde et al., 2012). Thus, studies reverse-engineering 5-HT_6 receptors are expected to highlight new therapeutic targets to co-opt disinhibition for the treatment memory deficits in disease, and inform novel therapy development for treatments of cognitive dysfunction in neurological diseases such as AD.

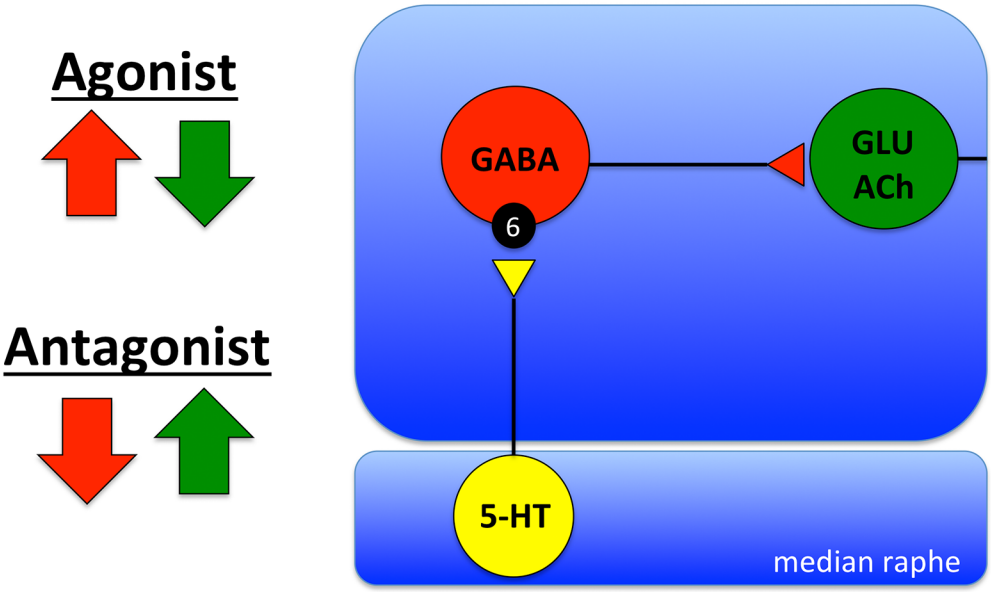


Figure 2.1. A working model of 5-HT_6 receptor-mediated disinhibition. Serotonergic fibers originating in the brainstem innervate inhibitory interneurons (GABA, red) throughout the brain thought to express 5-HT_6 receptors. Previous studies suggest 5-HT_6 receptor agonists increase the activity of GABAergic interneurons and thus, dampen the activity of glutamatergic or acetylcholinergic neurons. Conversely, 5-HT_6 receptor antagonists are thought to disinhibit glutamatergic (GLU, green) or acetylcholinergic (ACh, green) neurons by decreasing the activity of GABAergic neurons.

Figure 2.2. WAY-208466 attenuated the amplitudes of fEPSPs in the DG. A) Experimental schematic for recording fEPSPs in the DG. B) Representative fEPSPs taken at 60 min. The black and red traces are from ACSF- and WAY-208466-exposed slices, respectively. Scale bar: 0.2 mV, 5 ms. C) The amplitudes of the fEPSPs are normalized to the average amplitude of the last 4 fEPSPs during the baseline phase (18.5−20 min) and expressed as an average percentage ± SEM by condition. The black bar on top indicates when drug or ACSF was perfused onto the slices, and the numbers in parentheses indicate the number of slices tested from the number of animals. D) Time-course concentration-response of the effects of WAY-208466 on the amplitudes of fEPSPs in the DG. The average of the last 4 raw fEPSP amplitudes at the end of the drug phase (38.5−40 min) were not significantly attenuated compared to baseline for any concentration of WAY-208466 ($p > 0.05$, paired Student's *t* test). However, raw fEPSP amplitudes during the washout (58.5−60 min) were significantly attenuated compared to baseline for both 300 nM (green) and 1 μM (red) WAY-208466 (raw data not shown, * $p < 0.05$, paired Student's *t* test) E) Concentration-response for the washout phase. Each bar represents the mean amplitude during the washout (58.5−60 min) as a percent of baseline ± SEM in slices exposed to ACSF, 100 nM, 300 nM, or 1 μM WAY-208466. 1 μM WAY-208466 significantly attenuated the amplitudes of the fEPSPs compared to ACSF during the washout (** $p < 0.01$; one-way ANOVA with repeated measures and Bonferroni's multiple comparisons).

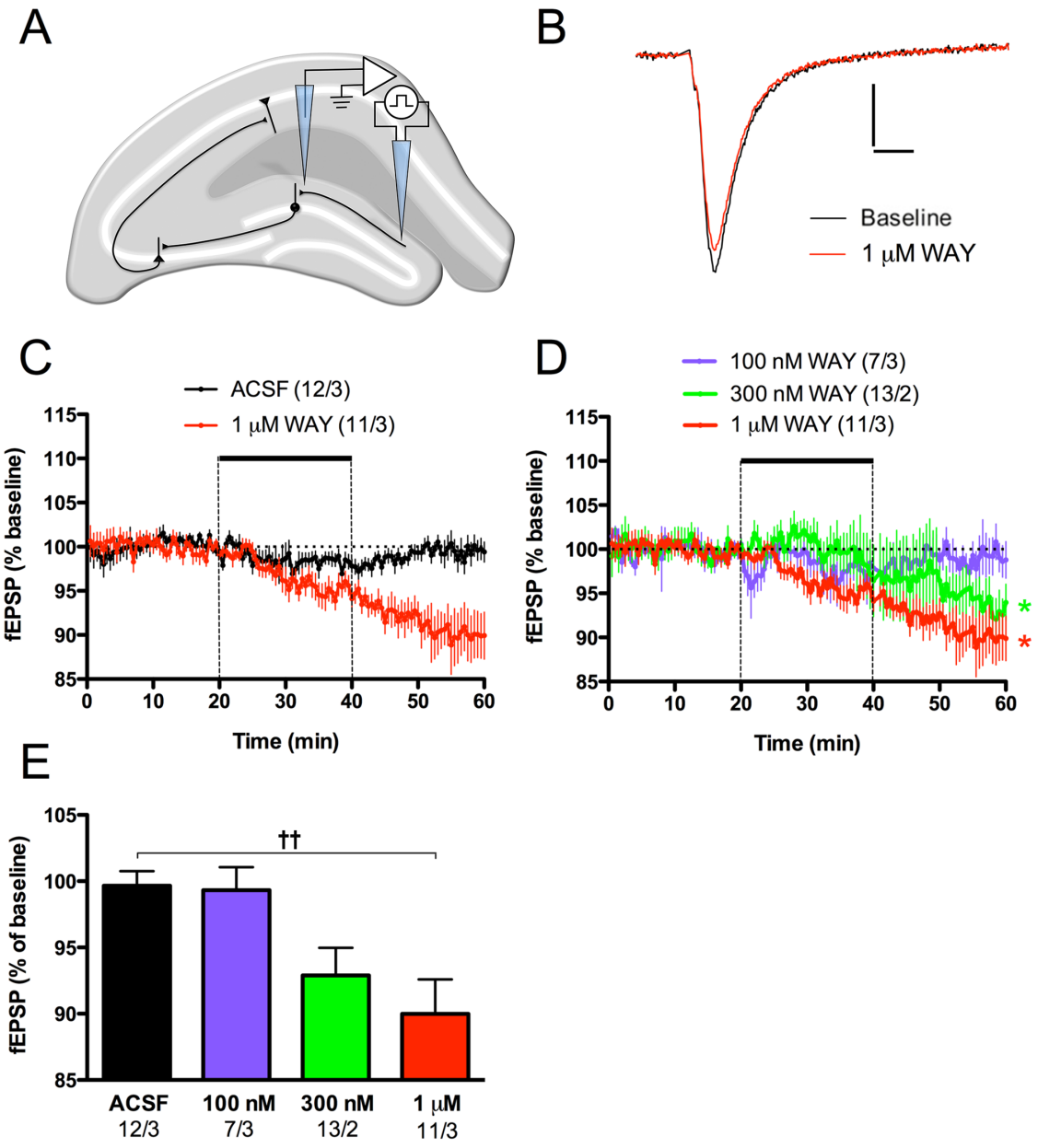
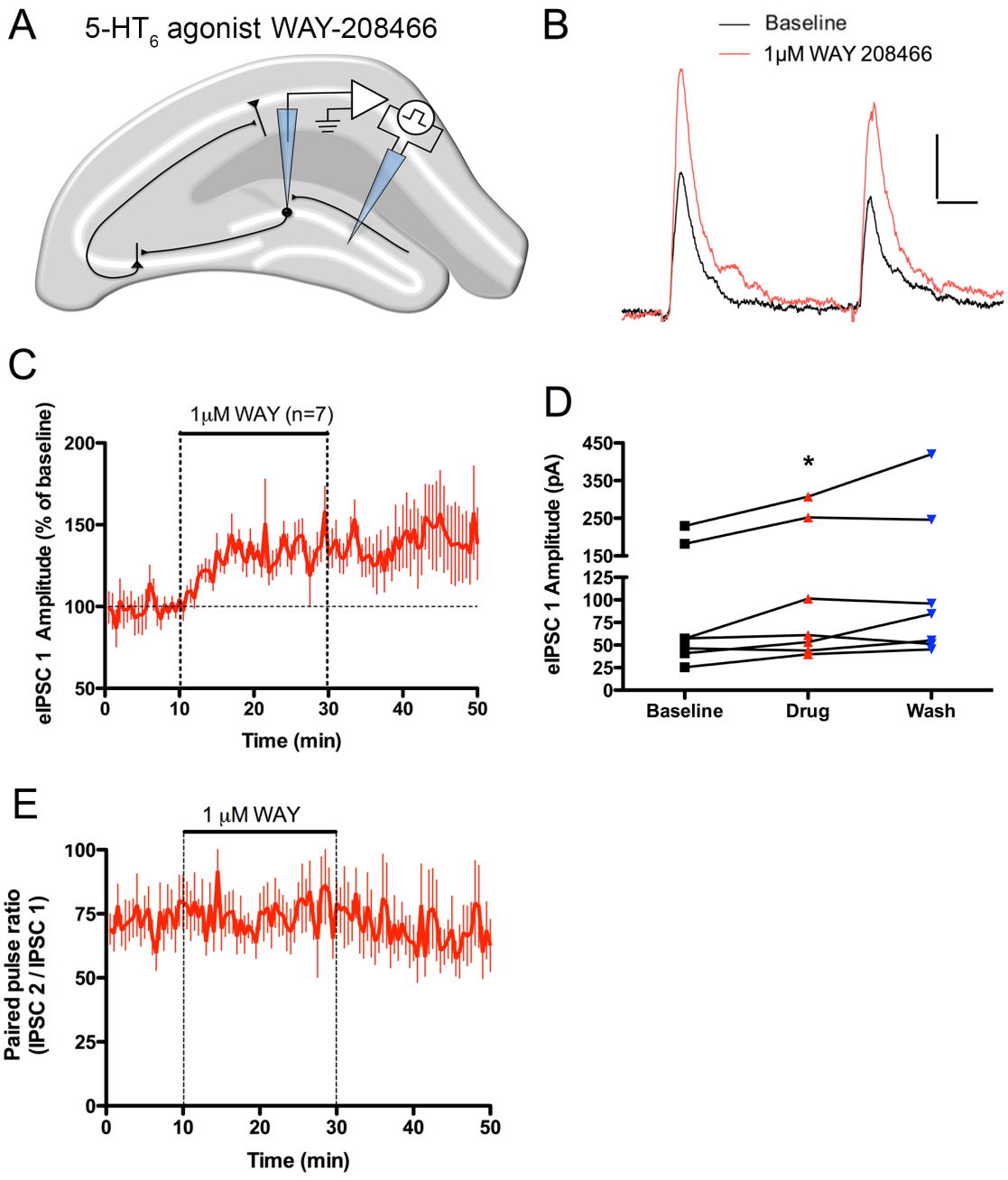

A
B
Baseline
1 μM WAY
C
ACSF (12/3)
1 μM WAY (11/3)
fEPSP (% baseline)
Time (min)
D
100 nM WAY (7/3)
300 nM WAY (13/2)
1 μM WAY (11/3)
fEPSP (% baseline)
Time (min)
E
fEPSP (% of baseline)
††
ACSF 12/3
100 nM 7/3
300 nM 13/2
1 μM 11/3

Figure 2.3. WAY-208466 attenuated the amplitudes of eIPSCs onto DGCs. A) Experimental schematic for recording eIPSCs in DGCs. B) Representative traces of paired-pulse eIPSCs during voltage-clamp recordings from DGCs before (black) and during (red) exposure to the 5-HT_6 receptor agonist WAY-208466 (1μM). Scale bars: 25 pA, 20 ms. C) Time course amplitudes of the first pulse (IPSC1) obtained during eIPSCs normalized to the average amplitude of the last 4 eIPSCs during the baseline phase (8.5−10 min) expressed as a percentage ± SEM. *n* = number of DGCs recorded from. D) WAY-208466 significantly increased the average raw amplitude of eIPSCs (* $p < 0.05$, Wilcoxon signed-rank test). E) There was no change in the PPR of eIPSCs over time ($p > 0.05$, Wilcoxon signed-rank test).


A
5-HT6 agonist WAY-208466
B
Baseline
1μM WAY 208466
C
1μM WAY (n=7)
eIPSC 1 Amplitude (% of baseline)
Time (min)
D
eIPSC 1 Amplitude (pA)
Baseline
Drug
Wash
E
1 μM WAY
Paired pulse ratio (IPSC 2 / IPSC 1)
Time (min)

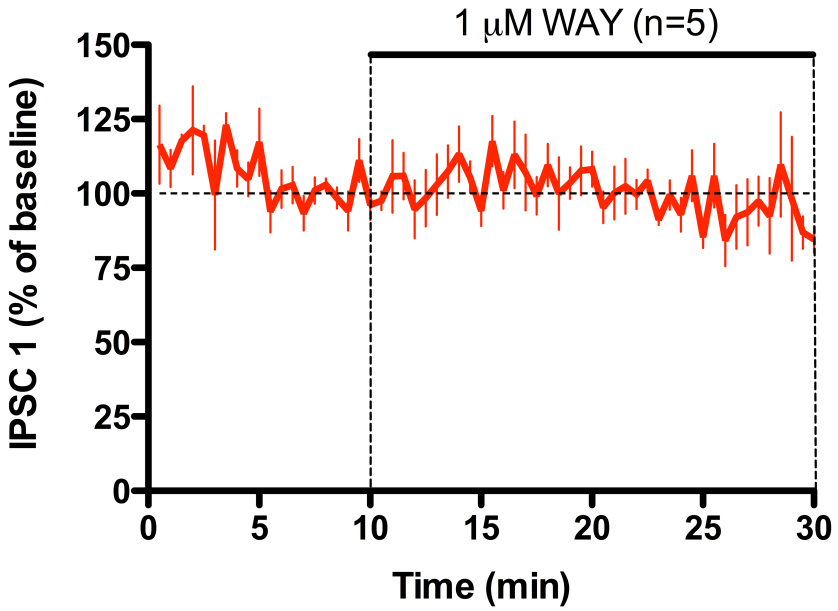


Figure 2.4. WAY-208466 no longer attenuated the amplitudes of eIPSCs onto DGCs. The amplitudes of the first pulse obtained during paired-pulse eIPSCs was normalized to the average amplitude of the last 4 eIPSCs during the baseline phase (8.5-10 min) and expressed as a percentage ± SEM. WAY-208466 no longer affected the amplitude of eIPSCs. See Figure 2.3 panel C for comparison.

Figure 2.5. SB-399885 attenuated the amplitudes of eIPSCs onto DGCs. A) Experimental schematic for recording eIPSCs from DGCs. B) Representative traces of paired-pulse eIPSCs during voltage-clamp recordings before (black) and during (red) exposure to the 5-HT_6 receptor antagonist SB-399885 (1μM). Scale bars: 50 pA, 25 ms. C) Time-course amplitudes of the first pulse obtained during eIPSCs (IPSC1) normalized to the average amplitude of the last 4 eIPSCs during the baseline phase (8.5−10 min) and expressed as a percentage ± SEM. The average normalized amplitude of eIPSC 1 at the end of the drug phase was significantly attenuated in SB-treated slices compared to ACSF-treated slices (††† $p < 0.001$, unpaired Student's t test). D) SB-399885 significantly attenuated the average raw amplitude of the eIPSC during the drug phase compared to the baseline phase (** $p < 0.01$, Wilcoxon signed-rank test), whereas ACSF had no significant effect on eIPSC amplitude (raw data for ACSF not shown, $p > 0.05$, paired Student's t test). E) Additionally SB-399885 did not affect the decay kinetics of eIPSC 1 ($n = 12$ (unable to obtain an accurate measurement of the decay kinetics for 1 cell), $p > 0.05$, paired Student's t test) or, F) eIPSC PPR ($p > 0.05$, paired Student's t test).

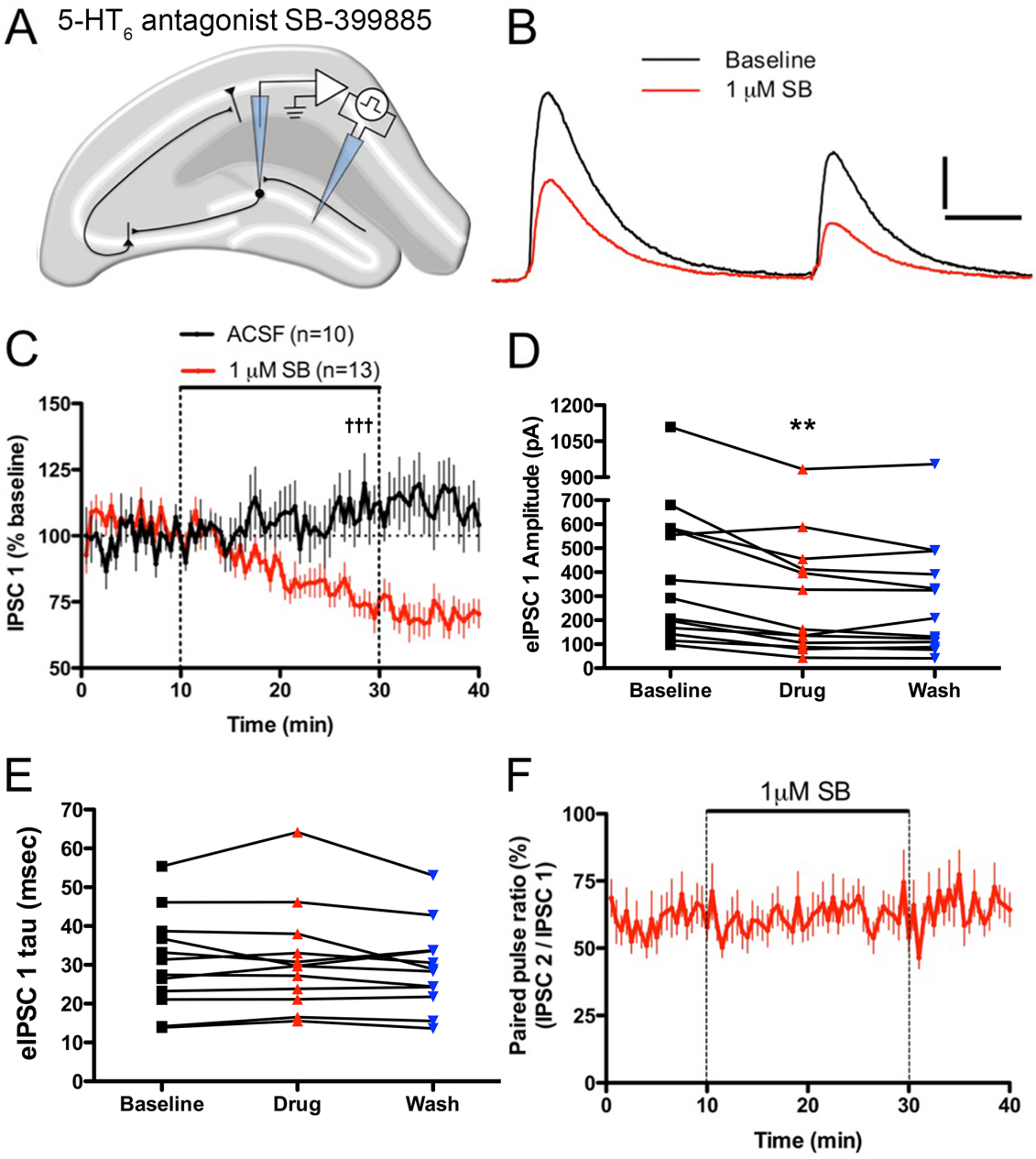

A
5-HT6 antagonist SB-399885
B
Baseline
1 μM SB
C
ACSF (n=10)
1 μM SB (n=13)
†††
IPSC 1 (% baseline)
Time (min)
D
**
eIPSC 1 Amplitude (pA)
Baseline
Drug
Wash
E
eIPSC 1 tau (msec)
Baseline
Drug
Wash
F
1μM SB
Paired pulse ratio (%)
(IPSC 2 / IPSC 1)
Time (min)

Figure 2.6. SB-399855 had no effect on the amplitude or IEI of sIPSCs during the drug phase, but increased IEI during the washout. A) Representative images of sIPSCs recorded from DGCs. Scale bars: 25 pA, 1 s. B) The amplitudes and (C) IEIs of sIPSCs are normalized to the average of the last 4 amplitudes and IEIs during the baseline phase (8.5−10 min) and expressed as a percentage ± SEM. IEI in SB-399885 treated slices was significantly increased compared to ACSF-treated slices during the washout phase only († $p < 0.05$, Welch's unequal variances t test). SB-399885 (1μM) had no affect on the average raw amplitudes (D) or IEIs (E) of SB-399885-treated slices during the drug phase. However, SB-399885-treated slices exhibited a delayed increase in the IEI during the washout phase compared to baseline (** $p < 0.01$, Wilcoxon signed-rank test).

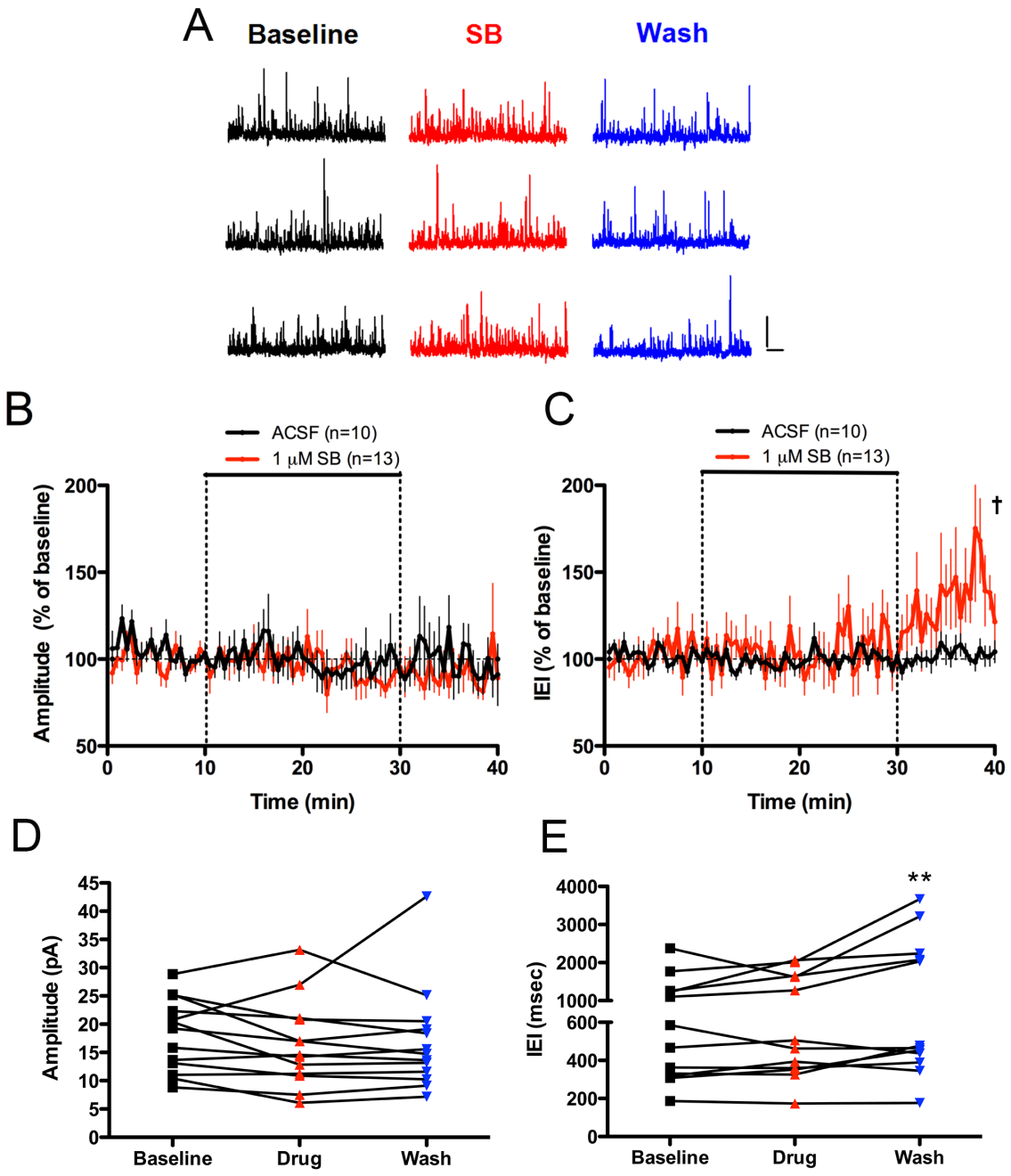

A
Baseline
SB
Wash
B
ACSF (n=10)
1 μM SB (n=13)
Amplitude (% of baseline)
Time (min)
C
ACSF (n=10)
1 μM SB (n=13)
IEI (% of baseline)
†
Time (min)
D
Amplitude (pA)
Baseline
Drug
Wash
E
IEI (msec)
**
Baseline
Drug
Wash

Figure 2.7. SB-399885 significantly attenuated the amplitudes of mIPSCs but did not affect their IEIs. A) Representative images of mIPSCs recorded from DGCs in the presence of 1 μM TTX. Scale bars: 25 pA, 1 s. B) The amplitudes and (C) IEIs of mIPSCs are normalized to the average of the last 4 amplitudes and IEIs during the baseline phase and expressed as a percentage ± SEM. D) The average raw mIPSC amplitudes and IEIs (E) are illustrated across by phase. SB-399885 significantly decreased the mean raw amplitudes of mIPSCs during the drug and washout phases but did not affect IEIs (** $p < 0.01$, paired Student's *t* test). Additionally, linear regression analysis of the normalized mIPSC amplitudes in panel B suggest that the slope of the line was significantly decreasing during all 3 phases including the baseline phase, and that SB-399885 did not significantly alter the slope of the line during the drug phase.

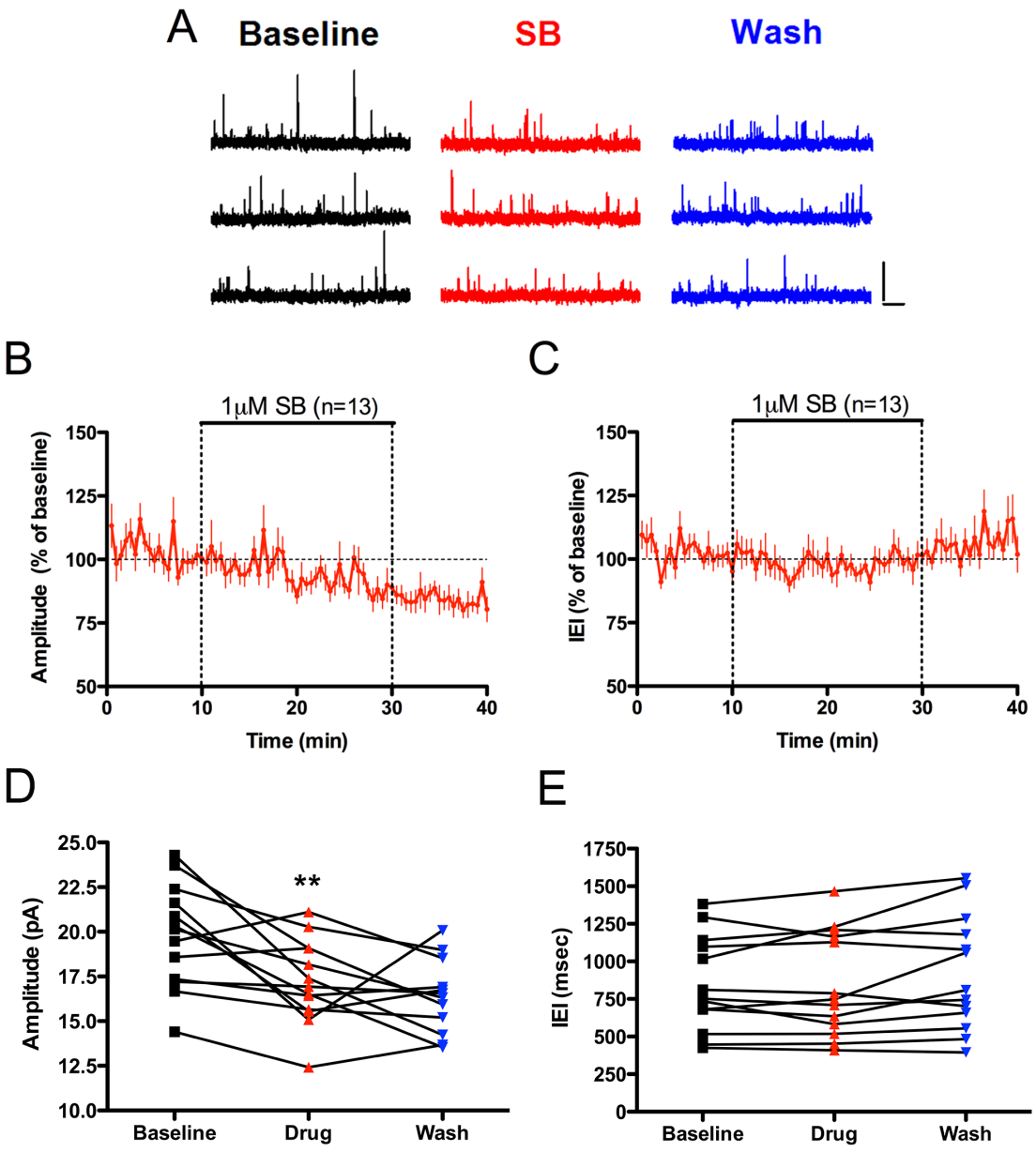

A
Baseline
SB
Wash
B
1μM SB (n=13)
Amplitude (% of baseline)
Time (min)
C
1μM SB (n=13)
IEI (% of baseline)
Time (min)
D
Amplitude (pA)
**
Baseline
Drug
Wash
E
IEI (msec)
Baseline
Drug
Wash

Figure 2.8. The majority of mossy cells in horizontally cut slices are GFP-IR. A) Absence of GFP staining in a horizontally cut section from a nontransgenic wild-type littermate. Note the lack of GFP filled cells in the hilus and granule cell layer. Scale bar, 100 μm. B) GFP staining in a horizontal section from an htr6-EGFP mouse. Note GFP filled cells in the hilus, and the occasional GFP staining in the granule cell layer indicated by arrows. Scale bar, 100 μm. C) Zoom in of the area indicated by the white box in panel B. Scale bar, 50 μm. Arrowheads indicate cells that are IR for GFP and, D) the mossy cell marker GluA2. Arrows indicate cells that are GluA2-IR but not GFP-IR. E) Arrows now indicate the presence of GABA filled cells, presumably inhibitory interneurons. F) Hoescht stain illustrating cellular nuclei. G_1) Zoom-in from panels C and D illustrating GFP and GluA2 merge. Arrowheads indicate cells co-labeled with GFP and GluA2 as illustrated by a yellow surround, presumably GFP filled mossy cells. Arrows indicate cells that are GluA2-IR but not GFP filled. Scale bar, 20 μm. G_2) Zoom-in from panels D and E. The arrow indicates a GABA-IR cell that does not express the mossy cell marker GluA2. H_1) Zoom-in from panels C and D illustrating another representative example of a GFP- and GluA2-IR cell (arrow head) and GluA2-IR cell that does not express GFP (arrow). H_2) Zoom-in showing a GABA-IR cell is not GluA2-IR.

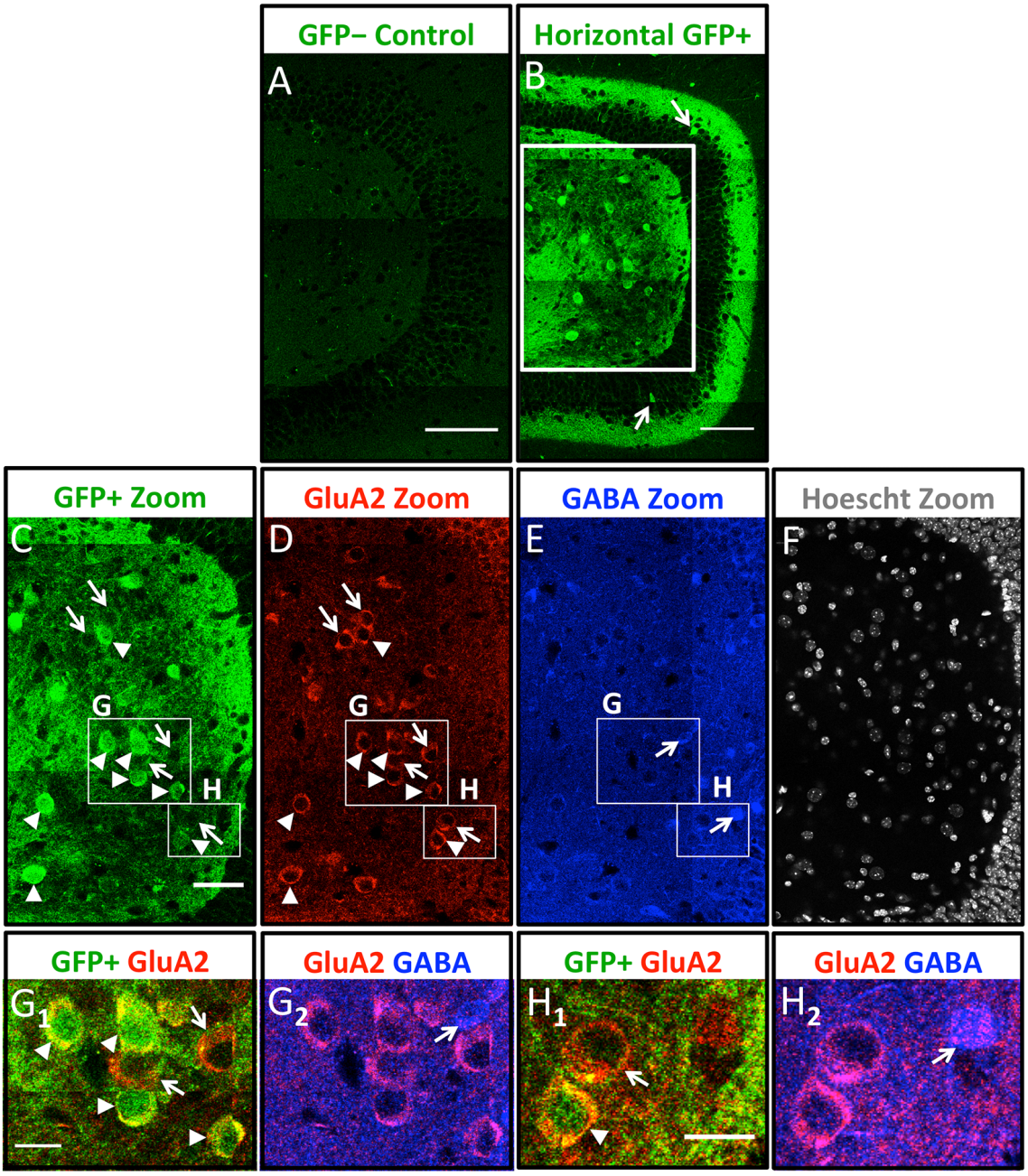
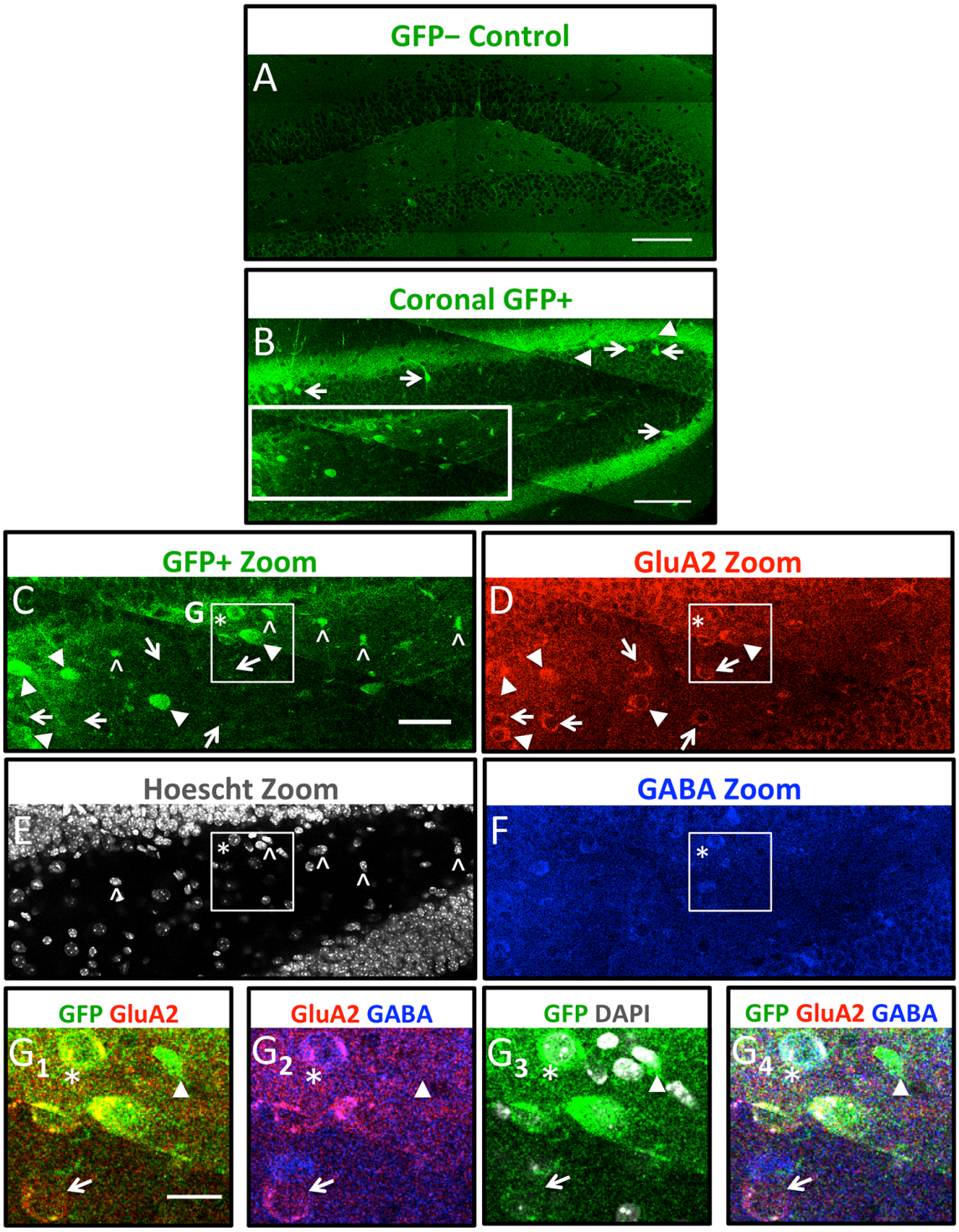

GFP– Control
A
Horizontal GFP+
B
GFP+ Zoom
C
GluA2 Zoom
D
GABA Zoom
E
Hoescht Zoom
F
G
H
GFP+ GluA2
G1
GluA2 GABA
G2
GFP+ GluA2
H1
GluA2 GABA
H2

Figure 2.9. The majority of mossy cells in coronally cut slices are GFP-IR. A) Absence of GFP staining in a coronally cut section from a nontransgenic wild-type littermate. Note the lack of GFP filled cells in the hilus and granule cell layer. Scale bar, 100 μm. B) GFP staining in a coronally cut section from an htr6-EGFP mouse. Note GFP filled cells in the hilus, and the occasional GFP staining in the granule cell layer indicated by arrows. C) Zoom in of the area indicated by the white box in panel B. Scale bar, 50 μm. Arrowhead indicates a cell that is IR for GFP and, D) the mossy cell marker GluA2. Arrows indicate cell that are GluA2-IR but not GFP-IR, presumably a mossy cell that does not express GFP. Carats indicate GFP-IR cells that are not GluA2-IR, E) but do have nuclei labeled with a Hoescht stain; possibly GFP-IR glial cells. Lastly, the asterisk indicates the rare occurrence of a GFP- and GluA2-IR cell, that is also, F) GABA-IR, presumably an inhibitory interneuron. G_1) Zoom-in from panels C and D illustrating GFP and GluA2 merge. Scale bar, 20 μm. Arrowhead indicates a GFP-IR cell that is co-labeled with GluA2 as illustrated by a yellow surround, presumably a GFP filled mossy cell. The arrow indicates a cell that is GluA2-IR but not GFP filled. The caret indicates a GFP-IR cell that is not, G_2) GluA2- or GABA-IR, G_3) but does have a nucleus. The asterisk indicates the rare occurrence of a cell that is IR for GFP, GluA2, and GABA, and has a nucleus. G_4) GFP, GluA2 and GABA merge illustrating all 3 markers in the cell above the asterisk.


GFP– Control
A
Coronal GFP+
B
GFP+ Zoom
C
G
GluA2 Zoom
D
Hoescht Zoom
E
GABA Zoom
F
GFP GluA2
G1
GluA2 GABA
G2
GFP DAPI
G3
GFP GluA2 GABA
G4

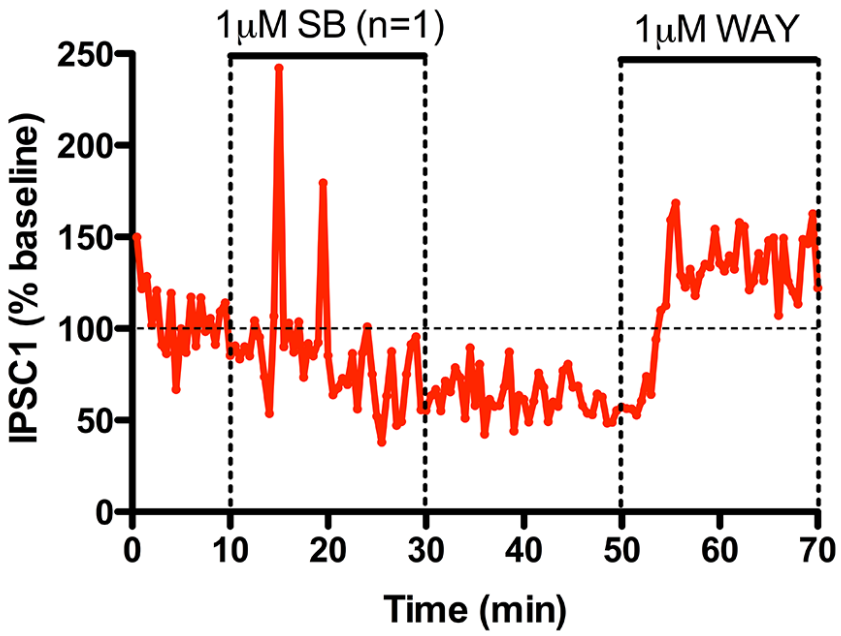


Figure 2.10. 5-HT_6 receptor ligands have similar effects in mouse and rat. The 5-HT_6 receptor antagonist SB-399885 attenuated the amplitudes of eIPSCs onto DGCs, and the 5-HT_6 receptor agonist enhanced the amplitudes of eIPSCs onto DGCs in a manner similar to that observed in hippocampal brains slices prepared from rat (n = 1) (see Figure 2.4 for comparison).

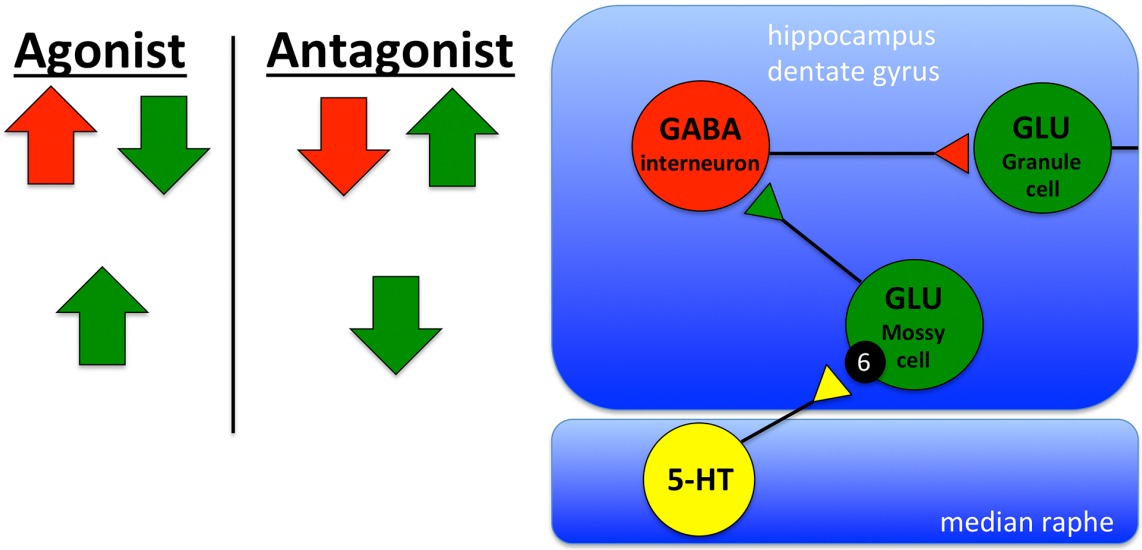


Figure 2.11. 5-HT_6 receptors indirectly modulate inhibitory synaptic transmission in the DG via mossy cells. Depicted on the right is a simplified schematic for synaptic connections in the DG. Together, our electrophysiology and IHC data suggests that 5-HT_6 receptor activation increases the activity of mossy cells (bottom, green) and their excitatory drive onto GABAergic inhibitory interneurons (red). This increases the activity of inhibitory interneurons that dampen the activity of granule cells (top, green). In contrast, our data also suggests that 5-HT_6 receptor antagonists directly attenuate the activity of mossy cells and thereby decrease the activity of inhibitory interneurons, thus increasing, or disinhibiting the activity of granule cells.

Table 2.1

GFP quantification in the DG of htr6-EGFP mice.

The number of mossy cells, granule cells, GABA+ cells, molecular layer (ML) cells, and other unknown cell types were either hand-counted or estimated (granule cells), and the percentage of each cell type IR for GFP is reported. We also quantified the mean percentage of each cell type that makes up total GFP expression in the DG. All averages are expressed ± SEM by the orientation of the slice. Horizontal, n = 6 slices from 2 animals. Coronal, n = 9 / 3.

	% of DG cell type that was GFP-IR		% of total GFP in the DG by cell type	
Cell type	Horizontal	Coronal	Horizontal	Coronal
Mossy Cells	81.1 ± 4.1	66.2 ± 4.4	65.6 ± 11.6	26.2 ± 2.9
Granule cells	1.2 ± 0.3	1.1 ± 0.1	29.2 ± 10.0	40.0 ± 3.4
GABA+ cells	0.3 ± 0.1	1.3 ± 0.4	0.3 ± 0.2	0.7 ± 0.3
ML cells	–	–	3.0 ± 1.6	15.1 ± 2.9
Other	–	–	1.9 ± 1.2	18.0 ± 2.3

References

Andel R, Hyer K, Slack A (2007) Risk factors for nursing home placement in older adults with and without dementia. J Aging Health 19:213-228.

Bonsi P, Cuomo D, Ding J, Sciamanna G, Ulrich S, Tscherter A, Bernardi G, Surmeier DJ, Pisani A (2007) Endogenous serotonin excites striatal cholinergic interneurons via the activation of 5-HT 2C, 5-HT6, and 5-HT7 serotonin receptors: implications for extrapyramidal side effects of serotonin reuptake inhibitors. Neuropsychopharmacology 32:1840-1854.

Boucher J, Mayes A, Bigham S (2012) Memory in autistic spectrum disorder. Psychol Bull 138:458-496.

Cembrowski MS, Wang L, Sugino K, Shields BC, Spruston N (2016) Hipposeq: a comprehensive RNA-seq database of gene expression in hippocampal principal neurons. Elife 5:e14997.

Cirillo MA, Seidman LJ (2003) Verbal declarative memory dysfunction in schizophrenia: from clinical assessment to genetics and brain mechanisms. Neuropsychol Rev 13:43-77.

Codony X, Vela JM, Ramirez MJ (2011) 5-HT(6) receptor and cognition. Curr Opin Pharmacol 11:94-100.

Da Silva Costa V, Duchatelle P, Boulouard M, Dauphin F (2009) Selective 5-HT6 receptor blockade improves spatial recognition memory and reverses age-related deficits in spatial recognition memory in the mouse. Neuropsychopharmacology 34:488-500.

Dawson LA, Nguyen HQ, Li P (2001) The 5-HT(6) receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. Neuropsychopharmacology 25:662-668.

Foley AG, Murphy KJ, Hirst WD, Gallagher HC, Hagan JJ, Upton N, Walsh FS, Regan CM (2004) The 5-HT(6) receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. Neuropsychopharmacology 29:93-100.

Fone KC (2008) An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. Neuropharmacology 55:1015-1022.

Froemke RC (2015) Plasticity of cortical excitatory-inhibitory balance. Annu Rev Neurosci 38:195-219.

Fujise N, Kosaka T (1999) Mossy cells in the mouse dentate gyrus: identification in the dorsal hilus and their distribution along the dorsoventral axis. Brain Res 816:500-511.

GENSAT Gene Expression Nervous System Atlas. Available at: http://gensat.org/index.html [Accessed: October 10, 2016].

Gerard C, el Mestikawy S, Lebrand C, Adrien J, Ruat M, Traiffort E, Hamon M, Martres MP (1996) Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. Synapse 23:164-173.

Gold CA, Budson AE (2008) Memory loss in Alzheimer's disease: implications for development of therapeutics. Expert Rev Neurother 8:1879-1891.

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N (2003) A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. Nature 425:917-925.

Goodrich-Hunsaker NJ, Hunsaker MR, Kesner RP (2008) The interactions and dissociations of the dorsal hippocampus subregions: how the dentate gyrus, CA3, and CA1 process spatial information. Behav Neurosci 122:16-26.

Grychowska K, Satala G, Kos T, Partyka A, Colacino E, Chaumont-Dubel S, Bantreil X, Wesolowska A, Pawlowski M, Martinez J, Marin P, Subra G, Bojarski AJ, Lamaty F, Popik P, Zajdel P (2016) Novel 1H-pyrrolo[3,2-c]quinoline nased 5-HT6 receptor antagonists with potential application for the treatment of cognitive disorders associated with Alzheimer's disease. ACS Chem Neurosci 7:972-983.

Hao J, Wang XD, Dan Y, Poo MM, Zhang XH (2009) An arithmetic rule for spatial summation of excitatory and inhibitory inputs in pyramidal neurons. Proc Natl Acad Sci U S A 106:21906-21911.

Helboe L, Egebjerg J, de Jong IE (2015) Distribution of serotonin receptor 5-HT6 mRNA in rat neuronal subpopulations: A double in situ hybridization study. Neuroscience 310:442-454.

Helmstaedter C (2002) Effects of chronic epilepsy on declarative memory systems. Prog Brain Res 135:439-453.

Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Medhurst AD (2003) Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol 64:1295-1308.

Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM, Riley G, Smith DR, Bartlett S, Heidbreder CA, Atkins AR, Lacroix LP, Dawson LA, Foley AG, Regan CM, Upton N (2006) SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol 553:109-119.

Hsu D (2007) The dentate gyrus as a filter or gate: a look back and a look ahead. Prog Brain Res 163:601-613.

Jiang X, Shen S, Cadwell CR, Berens P, Sinz F, Ecker AS, Patel S, Tolias AS (2015) Principles of connectivity among morphologically defined cell types in adult neocortex. Science 350:aac9462.

Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K (2012) Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron 76:1189-1200.

Kendall I, Slotten HA, Codony X, Burgueno J, Pauwels PJ, Vela JM, Fone KC (2011) E-6801, a 5-HT6 receptor agonist, improves recognition memory by combined modulation of cholinergic and glutamatergic neurotransmission in the rat. Psychopharmacology (Berl) 213:413-430.

King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KC (2004) 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation--an effect sensitive to NMDA receptor antagonism. Neuropharmacology 47:195-204.

Kohen R, Fashingbauer LA, Heidmann DE, Guthrie CR, Hamblin MW (2001) Cloning of the mouse 5-HT6 serotonin receptor and mutagenesis studies of the third cytoplasmic loop. Brain Res Mol Brain Res 90:110-117.

Kozlov AS, Angulo MC, Audinat E, Charpak S (2006) Target cell-specific modulation of neuronal activity by astrocytes. Proc Natl Acad Sci U S A 103:10058-10063.

Lacroix LP, Dawson LA, Hagan JJ, Heidbreder CA (2004) 5-HT6 receptor antagonist SB-271046 enhances extracellular levels of monoamines in the rat medial prefrontal cortex. Synapse 51:158-164.

Lee I, Hunsaker MR, Kesner RP (2005) The role of hippocampal subregions in detecting spatial novelty. Behav Neurosci 119:145-153.

Leranth C, Szeidemann Z, Hsu M, Buzsaki G (1996) AMPA receptors in the rat and primate hippocampus: a possible absence of GluR2/3 subunits in most interneurons. Neuroscience 70:631-652.

Letzkus JJ, Wolff SB, Luthi A (2015) Disinhibition, a circuit mechanism for associative learning and memory. Neuron 88:264-276.

Maher-Edwards G, Dixon R, Hunter J, Gold M, Hopton G, Jacobs G, Hunter J, Williams P (2011) SB-742457 and donepezil in Alzheimer disease: a randomized, placebo-controlled study. Int J Geriatr Psychiatry 26:536-544.

Maher-Edwards G, Zvartau-Hind M, Hunter AJ, Gold M, Hopton G, Jacobs G, Davy M, Williams P (2010) Double-blind, controlled phase II study of a 5-HT6 receptor

antagonist, SB-742457, in Alzheimer's disease. Curr Alzheimer Res 7:374-385.

Marazziti D, Baroni S, Pirone A, Giannaccini G, Betti L, Schmid L, Vatteroni E, Palego L, Borsini F, Bordi F, Piano I, Gargini C, Castagna M, Catena-Dell'osso M, Lucacchini A (2012) Distribution of serotonin receptor of type 6 (5-HT(6)) in human brain post-mortem. A pharmacology, autoradiography and immunohistochemistry study. Neurochem Res 37:920-927.

Marazziti D, Baroni S, Pirone A, Giannaccini G, Betti L, Testa G, Schmid L, Palego L, Borsini F, Bordi F, Piano I, Gargini C, Castagna M, Catena-Dell'osso M, Lucacchini A (2013) Serotonin receptor of type 6 (5-HT6) in human prefrontal cortex and hippocampus post-mortem: an immunohistochemical and immunofluorescence study. Neurochem Int 62:182-188.

Marcos B, Gil-Bea FJ, Hirst WD, Garcia-Alloza M, Ramirez MJ (2006) Lack of localization of 5-HT6 receptors on cholinergic neurons: implication of multiple neurotransmitter systems in 5-HT6 receptor-mediated acetylcholine release. Eur J Neurosci 24:1299-1306.

Millan MJ, Marin P, Bockaert J, Mannoury la Cour C (2008) Signaling at G-protein-coupled serotonin receptors: recent advances and future research directions. Trends Pharmacol Sci 29:454-464.

Mitchell ES, Hoplight BJ, Lear SP, Neumaier JF (2006) BGC20-761, a novel tryptamine analog, enhances memory consolidation and reverses scopolamine-induced memory deficit in social and visuospatial memory tasks through a 5-HT6 receptor-mediated mechanism. Neuropharmacology 50:412-420.

Myers CE, Scharfman HE (2009) A role for hilar cells in pattern separation in the dentate gyrus: a computational approach. Hippocampus 19:321-337.

Nygaard HB (2013) Current and emerging therapies for Alzheimer's disease. Clin Ther 35:1480-1489.

Pauls F, Petermann F, Lepach AC (2014) Episodic memory and executive functioning in currently depressed patients compared to healthy controls. Cogn Emot:1-18.

Paulus W, Rothwell JC (2016) Membrane resistance and shunting inhibition: where biophysics meets state-dependent human neurophysiology. J Physiol 594:2719-2728.

Raina P, Santaguida P, Ismaila A, Patterson C, Cowan D, Levine M, Booker L, Oremus M (2008) Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. Ann Intern Med 148:379-397.

Ramirez MJ (2013) 5-HT6 receptors and Alzheimer's disease. Alzheimers Res Ther 5:15.

Riemer C, Borroni E, Levet-Trafit B, Martin JR, Poli S, Porter RH, Bos M (2003)

Influence of the 5-HT6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a potent and selective 5-HT6 receptor antagonist. J Med Chem 46:1273-1276.

Rogers DC, Hagan JJ (2001) 5-HT6 receptor antagonists enhance retention of a water maze task in the rat. Psychopharmacology (Berl) 158:114-119.

Scharfman HE (1995) Electrophysiological evidence that dentate hilar mossy cells are excitatory and innervate both granule cells and interneurons. J Neurophysiol 74:179-194.

Scharfman HE (2007) The CA3 "backprojection" to the dentate gyrus. Prog Brain Res 163:627-637.

Scharfman HE, Sollas AL, Berger RE, Goodman JH (2003) Electrophysiological evidence of monosynaptic excitatory transmission between granule cells after seizure-induced mossy fiber sprouting. J Neurophysiol 90:2536-2547.

Schechter LE, Lin Q, Smith DL, Zhang G, Shan Q, Platt B, Brandt MR, Dawson LA, Cole D, Bernotas R, Robichaud A, Rosenzweig-Lipson S, Beyer CE (2008) Neuropharmacological profile of novel and selective 5-HT6 receptor agonists: WAY-181187 and WAY-208466. Neuropsychopharmacology 33:1323-1335.

Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81:741-766.

Setola V, Roth BL (2003) Why mice are neither miniature humans nor small rats: a cautionary tale involving 5-hydroxytryptamine-6 serotonin receptor species variants. Mol Pharmacol 64:1277-1278.

Skodzik T, Holling H, Pedersen A (2013) Long-term memory performance in adult ADHD: a meta-analysis. J Atten Disord [Epub ahead of print].

Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol Rev 99:195-231.

Stean TO, Hirst WD, Thomas DR, Price GW, Rogers D, Riley G, Bromidge SM, Serafinowska HT, Smith DR, Bartlett S, Deeks N, Duxon M, Upton N (2002) Pharmacological profile of SB-357134: a potent, selective, brain penetrant, and orally active 5-HT(6) receptor antagonist. Pharmacol Biochem Behav 71:645-654.

Stopps M, Allen N, Barrett R, Choudhury HI, Jarolimek W, Johnson M, Kuenzi FM, Maubach KA, Nagano N, Seabrook GR (2004) Design and application of a novel brain slice system that permits independent electrophysiological recordings from multiple slices. J Neurosci Methods 132:137-148.

Vicari S, Bellucci S, Carlesimo GA (2005) Visual and spatial long-term memory:

differential pattern of impairments in Williams and Down syndromes. Dev Med Child Neurol 47:305-311.

Wang HY, Lu CW, Lin TY, Kuo JR, Wang SJ (2016) WAY208466 inhibits glutamate release at hippocampal nerve terminals. Eur J Pharmacol 781:117-127.

Ward RP, Dorsa DM (1996) Colocalization of serotonin receptor subtypes 5-HT2A, 5-HT2C, and 5-HT6 with neuropeptides in rat striatum. J Comp Neurol 370:405-414.

West PJ, Marcy VR, Marino MJ, Schaffhauser H (2009) Activation of the 5-HT(6) receptor attenuates long-term potentiation and facilitates GABAergic neurotransmission in rat hippocampus. Neuroscience 164:692-701.

West PJ, Saunders GW, Remigio GJ, Wilcox KS, White HS (2014) Antiseizure drugs differentially modulate theta-burst induced long-term potentiation in C57BL/6 mice. Epilepsia 55:214-223.

Wilkinson D, Windfeld K, Colding-Jorgensen E (2014) Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Neurol 13:1092-1099.

Williams PA, Larimer P, Gao Y, Strowbridge BW (2007) Semilunar granule cells: glutamatergic neurons in the rat dentate gyrus with axon collaterals in the inner molecular layer. J Neurosci 27:13756-13761.

Woolley ML, Bentley JC, Sleight AJ, Marsden CA, Fone KC (2001) A role for 5-ht6 receptors in retention of spatial learning in the Morris water maze. Neuropharmacology 41:210-219.

Woolley ML, Marsden CA, Fone KC (2004) 5-ht6 receptors. Curr Drug Targets CNS Neurol Disord 3:59-79.

Wu Z, Guo Z, Gearing M, Chen G (2014) Tonic inhibition in dentate gyrus impairs long-term potentiation and memory in an Alzheimer's [corrected] disease model. Nat Commun 5:4159.

CHAPTER 3

5-HT_6 RECEPTOR ANTAGONISTS AND THEIR EFFECTS ON SPATIAL PATTERN PROCESSING AND SEIZURES IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

Abstract

Progressive loss of cognitive function in Alzheimer's disease (AD) may be partly driven by hyperexcitability in the form of seizures and subclinical epileptiform activity. However, current treatments for AD have seizure liabilities and are only marginally effective at improving cognitive function. Thus, procognitive and antiseizure treatments are needed. 5-HT_6 receptor antagonists have procognitive and anticonvulsant effects in rodents, and have been shown to improve cognitive function in patients with AD. However, their effects on seizures and cognition in a transgenic mouse model of AD are unreported, and may differ from those in otherwise healthy animals. We tested the hypothesis that 5-HT_6 receptor antagonists will exhibit procognitive and anticonvulsant effects in J20 mice, a transgenic model of familial AD that exhibits cognitive dysfunction and aberrant hyperexcitability. J20 mice were first evaluated in a spatial pattern processing task reliant on proper DG-function – the metric task. Treatment with a 5-HT_6 receptor antagonist SB-399885 (10 mg / kg, intraperitoneal (i.p.)) 30 min prior to testing

improved performance in nontransgenic (NTG) but not J20 mice, and also attenuated distance traveled in both genotypes. Additionally, vehicle-treated J20 mice exhibited a significantly lower seizure threshold than vehicle-treated NTG mice, and SB-399885 (30 mg / kg, i.p.) had no effect on either genotype's seizure threshold. We also tested the hypothesis that SB-399885 and another 5-HT_6 receptor antagonist, SB-271046, (both 10 mg / kg, i.p.) would exhibit anticonvulsant effects in naïve C57BL/6 and CF1 male mice in the minimal clonic seizure threshold test. Surprisingly, SB-399885 reduced seizure threshold in C57BL/6 mice but had no effect on seizure threshold in CF1 mice. SB-271046 had no effect on seizure threshold in either strain. These results suggest 5-HT_6 receptor antagonists significantly improved DG-associated spatial pattern processing in NTG mice but not J20 mice, and that 5-HT_6 receptor antagonists have strain- and ligand dependent proconvulsant effects in naïve and otherwise healthy mice. Thus, 5-HT_6 receptor antagonists exhibited varying effects on cognition and seizures and should be more thoroughly evaluated, particularly with respect to their effects on seizures, to inform their current clinical use in humans with AD that have an increased tendency for seizures.

Introduction

Alzheimer's disease (AD) is a progressive neurological disorder beginning with memory deficits that slowly advances to a complete loss of cognitive function. As of 2015, 5.4 million Americans suffer from AD, and it is estimated that number will triple by 2050 (Alzheimer's Association, 2015). Annual healthcare costs of AD, primarily arising from round-the-clock nursing care, exceeds $600 billion and places an enormous financial burden on patients, their families, and society (Wimo et al., 2013). Memory

impairment is among the primary causes for admission into residential nursing care (Gaugler et al., 2009), and it is estimated that delaying nursing home admission for all dementia patients in the U.S. by only 1 month would cut spending by $4 billion (Gold and Budson, 2008). However, current treatments for memory impairment in AD are few in number, mechanistically homogenous, and only marginally effective (Raina et al., 2008; Nygaard, 2013). Thus, new treatments that attenuate memory deficits will improve quality of life and diminish the heavy financial and emotional burden of AD.

Recent studies have highlighted that in addition to cognitive dysfunction, anywhere from 1.5–64% of AD patients have had at least 1 unprovoked seizure, with particularly high rates in patients with familial AD (FAD) (Friedman et al., 2012). Patients with AD and epilepsy exhibit greater cognitive impairments and their symptoms typically progress faster than AD patients without epilepsy (McAreavey et al., 1992; Volicer et al., 1995). Moreover, it is suspected that up to half of all AD patients with epilepsy have nonconvulsive seizures, and 43% exhibit subclinical epileptiform activity (Vossel et al., 2013; Vossel et al., 2016). These studies suggests that a large portion of AD patients may be having "silent seizures" and bursts of hypersynchronous discharges that disrupt their excitation-inhibition balance and cognition (Kleen et al., 2013). Moreover, transgenic animal models of AD, patients with mild cognitive impairment, and even patients with probable AD have been shown to exhibit hyperexcitability and cognitive impairments that are attenuated by the anticonvulsant drug levetiracetam (LEV) (Cumbo and Ligori, 2010; Bakker et al., 2012; Sanchez et al., 2012). Thus, hyperexcitability is now considered an early pathological mechanism in AD, and new treatment approaches for AD need to address *both* cognitive dysfunction and seizures.

Treating cognitive dysfunction in the presence of pathological hyperexcitability presents a double-edged sword: many cognition-enhancing drugs have seizure liabilities, and seizures and subclinical epileptiform activity disturb memory, whereas many anticonvulsant drugs dampen seizures, but also worsen cognitive function. For example, 2 of the 5 currently approved drug treatments for cognitive dysfunction in AD, Aricept® (Donepezil) and Exelon® (Rivastigmine), are listed on the World Health Organization's top ten drugs associated with convulsive adverse drug reactions (Kumlien and Lundberg, 2010). Memantine, the only approved treatment for cognitive dysfunction in AD that is not an acetylcholinesterase inhibitor, comes with a package insert warning for seizures, is associated with new-onset seizures in AD (Peltz et al., 2005), and worsens seizures in animal models of epilepsy (Loscher, 1998; Mares and Mikulecka, 2009). On the other hand, several first and second generation antiseizure drugs (ASDs) that are effective for reducing seizures have been shown to worsen cognitive function in healthy volunteers, patients with epilepsy, as well as AD patients (Meador et al., 1993; Meador et al., 1995; Meador, 2002; Lee et al., 2003; Meador et al., 2005; Salinsky et al., 2005; Hamed, 2009; Cumbo and Ligori, 2010). Thus, most currently prescribed ASDs are not well suited to treat seizures in AD. Although LEV reduced seizures and cognitive dysfunction in animals models and patients with AD (Cumbo and Ligori, 2010; Bakker et al., 2012; Sanchez et al., 2012), and Phase II clinical trials evaluating the cognitive effects of LEV in AD are currently underway are still needed, particularly those that may exhibit improved efficacy and lack side effects associated with LEV, such as aggression and irritability (Helmstaedter et al., 2013).

One class of drugs that may improve cognitive function without worsening

seizures is serotonin (5-HT) 5-HT_6 receptor antagonists. Drugs that block the activity of 5-HT_6 receptors have been shown to improve memory in naïve, aged, and amnestic rodents (Rogers and Hagan, 2001; Woolley et al., 2001; Stean et al., 2002; Foley et al., 2004; King et al., 2004; Hirst et al., 2006; Mitchell et al., 2006; Da Silva Costa et al., 2009; Kendall et al., 2011), and have even been shown to improve cognitive function in randomized, double-blind, placebo controlled clinical trials in patients with mild-to-moderate probable AD as an adjunctive therapy to Donepezil (Maher-Edwards et al., 2011; Wilkinson et al., 2014). In addition to their procognitive effects, 5-HT_6 receptor antagonists also exhibit anticonvulsant effects. For instance, several 5-HT_6 receptor antagonists have been shown to increase seizure threshold in rats during the maximal electroshock test, (Routledge et al., 2000; Hirst et al., 2006), and 5-HT_6 receptor blockade produced a reduction in seizure severity and latency when administered prior to pilocarpine treatment in rats (Wang et al., 2015). Despite their advancement to clinical trials in humans, their effects on cognition and seizures have never been reported in an animal model of FAD that exhibits spontaneous seizures. Understanding their effects in a relevant disease model of AD is necessary because their effects on cognition and seizures may differ from those in otherwise healthy animals due to serotonergic remodeling (Garcia-Alloza et al., 2004; Ramirez et al., 2014). More specifically, the effects of 5-HT_6 receptor antagonists on seizures have only been minimally evaluated and could have beneficial effects on 1 type of seizure and deleterious effects on another – effects that could easily go unnoticed in patient populations. Thus, additional studies are needed to comprehensively test the effects of 5-HT_6 receptor antagonists on cognition and seizures in AD.

Therefore, we tested the effects of a 5-HT_6 receptor antagonist on cognitive function and seizures in J20 mice – a transgenic model of FAD that expresses human amyloid precursor protein (hAPP) harboring Swedish and Indiana mutations (Mucke et al., 2000). At 3- to 5-months-of-age, J20 mice accumulate amyloid-beta plaques and develop spontaneous nonconvulsive seizures, hippocampal-dependent memory deficits, and impaired synaptic plasticity, as well as increased GABAergic synaptic transmission in the dentate gyrus (DG) (Palop et al., 2007) thought to be a compensatory response to hyperexcitability. Thus, treatments that dampen excess inhibition in the DG of J20 mice without exacerbating seizures may restore normative DG physiology and function in J20 mice. Towards this end, 5-HT_6 receptor antagonists are thought to produce their nootropic effects, in part, by dampening inhibitory synaptic transmission to facilitate information flow through excitatory networks – a phenomenon known as disinhibition (Dawson et al., 2001; Fone, 2008; Schechter et al., 2008; West et al., 2009; Codony et al., 2011). Moreover, work in Chapter 2 of this dissertation revealed that 5-HT_6 receptors bidirectionally control inhibitory synaptic transmission in the DG in a manner consistent with 5-HT_6 receptor antagonist-mediated disinhibition. Thus, 5-HT_6 receptor antagonists' established procognitive and anticonvulsant effects may be subtle enough to attenuate aberrantly increased inhibition in the DG of J20 mice and improve DG-mediated cognitive function without exacerbating seizures.

We hypothesized that the 5-HT_6 receptor antagonist SB-399885 exhibits procognitive and anticonvulsant effects in J20 mice. We also tested the hypothesis that 5-HT_6 receptor antagonists would exhibit anticonvulsant effects in 2 strains of naïve and otherwise healthy mice. First, J20 mice were evaluated for spatial pattern processing

deficits in the metric task, a behavioral paradigm that relies on proper DG function (Ennaceur and Delacour, 1988; Lee et al., 2005; Goodrich-Hunsaker et al., 2008; Ennaceur, 2010). Preliminary data suggested J20 mice may be impaired in the metric task. Therefore, we tested the effects of acute treatment with SB-399885 in J20 mice and their NTG littermates. Following cognitive testing, we also evaluated the effects of 5-HT_6 receptor blockade on seizure threshold in the minimal clonic seizure threshold test in J20 and NTG mice, as well as 2 additional strains of naïve and otherwise healthy mice.

Methods

Animals

Four- to six-month-old (20–25 g) male heterozygous transgenic J20 mice expressing human amyloid precursor protein (hAPP) containing the Swedish and Indiana FAD mutations, as well as their nontransgenic (NTG) wild-type littermates, were obtained from Jackson Laboratory, Mutant Mouse Regional Research Center (Mucke et al., 2000). Three cohorts of J20 and NTG mice were purchased. J20: n = 22, NTG: n = 24. The first cohort was used for minimal clonic seizure threshold testing (n = 8 per group) and the second and third cohorts were used for cognitive testing in the metric task (J20: n = 14; NTG: n = 16).

Seven- to eight-week-old male C57BL/6 mice (20–25 g, Charles River Laboratories, Raleigh, NC, U.S.A.) and 5- to 6-week-old male CF-1 mice (18–25g; Charles River Laboratories, Wilmington, MA) were used to test the effects of 5-HT_6 receptor antagonists on minimal clonic seizure threshold in naïve mice. The number of mice used in each experiment is indicated in each figure. All mice were group housed in

a light- and temperature-controlled (12 h on/12 h off) environment and permitted access to food and water *ad libitum* throughout the study. All experiments were conducted during the light cycle, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and approved by the University of Utah Institutional Animal Care and Use Committee. All efforts were made to minimize the number and suffering of animals used.

Metric Task

Beginning at 4- to 6-months of age (20–25 g), J20 mice and their NTG littermates were tested in the metric task. Mice were acclimated to the testing room for 1 h prior to behavioral testing. Mice were then individually habituated to a square plexiglass arena (40 L x 40 W x 60 H cm; illumination: ~50 lux) for 2 consecutive days by freely exploring the testing arena for 10 min. Animals were then placed into an empty holding cage for 5 min, and returned to the arena for an additional 5 min of exploration. During habituation, the arena contained 2 objects in separate locations that were not used during testing and were not moved during habituation. Distinct visual spatial cues were always located 15 cm from each side of the arena.

On day 3, each mouse was weighed and administered the 5-HT_6 receptor antagonist SB-399885 (10 mg/kg, i.p.) or vehicle (0.5% methyl cellulose with 0.2% Tween, i.p.) 30 min prior to testing, which was conducted in 5 phases (see Figure 3.1A for an experimental schematic of the metric task). During phase 1 (acquisition 1), mice were individually allowed to explore the arena containing 2 dissimilar objects placed 30 cm apart for 5 min. Mice were then moved to a holding cage for 5 min (phase 2, delay 1)

while the arena underwent a minimal cleaning protocol to wipe up any feces, and were then placed back into the arena for 15 min (phase 3, acquisition 2). In phase 4 (delay 2), the animal was removed from the arena and placed in a holding cage for 5 min while the arena underwent minimal cleaning. During this time, the objects were replaced with a duplicate, precleaned pair of objects now placed 8 cm apart. During phase 5 (test), the mouse was placed back in the arena for an additional 5 min. Before each trial, the arena and 4 objects (a duplicate pair of dissimilar objects) were precleaned with a 0.4% HDQ solution.

During testing, animals were tracked using EthoVision software (Noldus, Leesburg, VA, U.S.A.). An observer who was "blinded" to the experimental group manually scored object exploration. Exploration was defined as investigative behavior, where the mouse was orienting its head towards the object when within 2 cm of the object, or touching or smelling the object with its nose, whiskers or forepaws. A mouse that was climbing on the objects, or in close proximity without actively attending to it, was not counted as exploration. Throughout the acquisition and test phases, total object exploration time, regardless of which object was being explored, was recorded in 5-min epochs. Performance was then expressed as a recognition index (RID):

$$\frac{(\text{object exploration})_{\text{test}} - (\text{object exploration})_{\text{acquisition 10–15 min}}}{(\text{object exploration})_{\text{test}} + (\text{object exploration})_{\text{acquisition 10–15 min}}}$$

RIDs were calculated for each mouse and averaged by experimental group. Total distance traveled (cm), time spent in the center, and number of center crossings normalized to individual distance traveled were measured using EthoVision and averaged by treatment group. Data are expressed as the mean ± standard error of the mean (SEM) with scatter plots. Data from crossover studies are presented as paired scatter plots.

First, J20 and NTG mice (n = 6 per genotype) were tested in a pilot study in which they were injected with vehicle only 30 min prior to the beginning of phase 1. One week following this preliminary study, we tested the effects of the 5-HT_6 receptor antagonist SB-399885 (10 mg / kg, i.p.) in J20 and NTG mice using a "crossover" design, such that animals treated with SB-399885 during the first week of testing were retested the following week after treatment with vehicle, and animals treated with vehicle during the first week of testing were retested the following week after drug treatment. We tested a second cohort of J20 (n = 14) and NTG (n = 16) mice in the metric task using a similar crossover experimental paradigm. New objects were used for the second half of each crossover so that mice never encountered the same objects when re-tested in the metric task.

Minimal Clonic Seizure Threshold Test

Dr. Peter J. West conducted minimal clonic seizure experiments in J20 mice. The author of this manuscript, Dr. Gregory J. Remigio, conducted minimal clonic seizure experiments in naïve C57BL/6 and CF1 mice.

Electrical stimulation during seizure testing was done using transcorneal stimulation with a custom constant current (60 Hz) electroconvulsometer (60 Hz, 0.2 ms sinusoidal current pulse, varying intensities). Prior to stimulation, 0.5% tetracaine was applied to the cornea for anesthesia and electrical conductivity. J20 mice and their NTG littermates (n = 8 per genotype) were individually administered the 5-HT_6 receptor antagonist SB-399855 (30 mg / kg, i.p.) or vehicle (0.5% methyl cellulose with 0.2% Tween, i.p.). Thirty minutes after drug treatment, convulsive current (CC) curves were

generated in J20 and NTG mice using a staircase procedure, such that stimulation intensity increased or decreased depending whether the previous stimulus produced a seizure. Minimal clonic seizures are characterized by rhythmic face and forelimb clonus and ventral neck flexion that may progress to rearing and falling. Behavioral seizures were visually confirmed by an observer who was "blinded" to the experimental groups. Due to a limited number of animals, minimal clonic seizure testing in J20 mice was completed across 4 experimental days each separated by 1 week using a "double crossover" design, such that each animal was treated with drug or vehicle twice. CC curves are illustrated with the percentage of mice that had a seizure at each stimulation strength tested. Data are also expressed as the CC_{50} by experimental group

In a separate group of experiments, naïve C57BL/6 and CF1 mice were administered SB-399885 (10 mg / kg, i.p.), SB-271046 (10 mg / kg i.p.), or vehicle (0.5% methyl cellulose with 0.2% Tween, i.p.). Thirty minutes later, mice were stimulated at their known CC_{50} (5.5 mA for C57BL/6 and 6.8 mA for CF1 mice), which were previously determined using the staircase method. Behavioral seizures were visually confirmed by an observer who was "blinded" to the treatment groups. Data are represented as the total number of mice to exhibit a seizure compared to the number of mice that did not have a seizure. Naïve mice were also tested using a "crossover" experimental design, with experimental days separated by at least 4 days. Minimal clonic seizure threshold data in naïve mice is expressed as the number of mice to exhibit a seizure versus the number of mice to not exhibit a seizure by experimental group.

Drug Preparation and Treatment

The 5-HT_6 receptor antagonists SB-399885 and SB-271046 were purchased from Tocris Bioscience (Minneapolis, MN, U.S.A.). All drugs were dissolved in 0.5% methyl cellulose with 0.2% tween (vehicle) to a stock concentration of 3 mg / mL or 1 mg / mL and were administered at either 30 mg / kg or 10 mg / kg, respectively, via i.p. injection 30 min prior to testing. Drugs for cognitive testing were made fresh at the beginning of the test week. Drugs for seizure testing were made fresh before each experiment.

Statistics

Metric task data obtained in the presence of vehicle injections alone were compared using an unpaired Student's *t* test. All metric task data obtained in the presence of drug or vehicle injection was compared using two-way repeated measures ANOVA with Bonferroni's post hoc test. For minimal clonic seizures threshold testing, CC curves generated in J20 and NTG mice were tested for statistical significance using Probit analysis (Minitab, Minitab Inc., State College, PA, U.S.A.). The mean CC_{50} in vehicle- and drug-treated J20 and NTG mice was compared using two-way repeated measures ANOVA with Bonferroni's post hoc test. Minimal clonic seizure threshold data obtained in naïve C57BL/6 or CF1 mice was compared using Fisher's exact test. All statistical analysis besides Probit was performed using GraphPad Prism V5.0c (GraphPad Software, San Diego, CA); $p < 0.05$ was considered significant. *, **, or *** indicate a *p*-value of < 0.05, < 0.01, and < 0.001.

Results

Performance of J20 Mice in the Metric Task

To determine whether J20 mice exhibit deficits during spatial pattern processing, we conducted a pilot study in which J20 mice and their NTG littermates were injected with vehicle only 30 min prior to testing in the metric task (Figure 3.1A, $n = 6$ per genotype); no drug was tested during this preliminary study. Results showed that the mean RIDs for J20 and NTG mice were not significantly different (Figure 3.1B, RID in NTG: 0.30 ± 0.11, J20: -0.01 ± 0.12, $p = 0.08$, unpaired Student's t test). A power analysis using variances generated in this experiment suggested the experiment was underpowered and that 8 animals per treatment group would have sufficiently powered the experiment. We also measured object exploration time in 5-min epochs, and showed that NTG mice spent more time exploring the objects during the test phase compared to the last 5 min of acquisition (Figure 3.1C, object exploration time, NTG test phase: 14.2 ± 1.1 s; last 5 min of acquisition: 8.5 ± 1.9 s, $p < 0.05$, paired Student's t test), whereas J20 mice did not spend significantly more time exploring the objects ($p > 0.05$, paired Student's t test). We also observed no significant difference in total object exploration time or total distance traveled between J20 and NTG mice (Figure 3.1D, E, $p > 0.05$, unpaired Student's t test). Together, these results suggest that J20 mice did not exhibit spatial pattern processing deficits during our preliminary experiment but may have exhibited spatial pattern processing deficits compared to NTG mice in an adequately powered experiment.

The Effects of SB-399885 on Performance of NTG and J20 Mice in the Metric Task

Next, we tested the effects of the 5-HT_6 receptor antagonist SB-399885 (10 mg / kg, i.p.) on spatial pattern processing in J20 and NTG mice in the metric task using a "crossover" experimental design (n = 6 per treatment group). Two-way repeated measures ANOVA revealed no significant effect of treatment group, genotype, or interaction (Figure 3.2A, Treatment group: $F_{(1, 10)} = 2.79$; $p = 0.13$). Despite poor performances by vehicle-treated NTG and J20 mice (an average RID of 0.30 is common for healthy animals in novelty recognition tasks (Smith et al., 2014)), the data suggested that SB-399885 improved performance in *both* NTG and J20 mice. Therefore, we tested a second cohort of J20 and NTG mice (n = 8 and 10, respectively) in the metric task following treatment with SB-399885 (10 mg / kg, i.p.). Two-way repeated measures ANOVA revealed a significant effect of treatment but not genotype or interaction (Figure 3.2B, $F_{(1, 16)} = 7.43$; $p < 0.05$). Bonferroni's post hoc test revealed this effect was significant in NTG mice only (RID Vehicle: -0.04 ± 0.07; SB-399885: 0.33 ± 0.12; $p < 0.05$). We also combined data from the first and second cohorts that were treated with vehicle or SB-399885 (Combined, J20: n = 14; NTG: n = 16). Similarly, two-way repeated measures ANOVA revealed a significant effect of treatment but not for genotype or interaction (Figure 3.2C, $F_{(1, 28)} = 10.18$; $p < 0.01$). Bonferroni's post hoc test revealed this effect was significant in NTG mice only (RID Vehicle: 0.01 ± 0.07; SB-399885: 0.32 ± 0.09; $p < 0.05$). In summary, testing in 2 cohorts of J20 mice and their NTG littermates suggests that the 5-HT_6 receptor antagonist SB-399885 improved performance during the metric task in NTG mice only.

To better understand these results, we also quantified total distance traveled, time spent in the center, number of center crossing normalized to distance traveled, and total object exploration time for the combined cohorts of J20 mice and their NTG littermates during the metric task (Figure 3.3). Two-way repeated measures ANOVA showed a significant effect of treatment on total distance traveled, but no effect of genotype or interaction (Figure 3.3A; $F_{(1,28)} = 47.67$; $p < 0.001$). Bonferroni's post hoc test revealed SB-399885-treated NTG mice traveled significantly shorter distances than vehicle-treated NTG mice (NTG VEH: 6175 ± 440 cm; SB: 4339 ± 191 cm, $p < 0.001$). The same trend was observed in J20 mice (J20 VEH: 6202 ± 483 cm; VEH: 4228 ± 536 cm $p < 0.001$). Additionally, SB-399885 significantly attenuated total time spent in the center (Figure 3.3B; $F_{(1,28)} = 6.76$; $p < 0.05$, two-way repeated measures ANOVA). However, Bonferroni's multiple comparisons revealed no significant differences between vehicle- or SB-399885-treated mice in either genotype. There were no significant effects observed on total number of center entries normalized to 1000 cm of distance traveled (Figure 3.3C, $p > 0.05$, two-way repeated measures ANOVA). Together, these results suggest that SB-399885 may have had sedative and / or anxiogenic effects in NTG and J20 mice. However, the reduction in distance traveled was not due to increased time spent exploring the objects since we observed a significant decrease, rather than an increase, in total object exploration time in SB-treated animals (Figure 3.3D; $F_{(1,28)} = 8.37$; $p < 0.01$, two-way repeated measures ANOVA). Bonferroni's multiple comparisons revealed no significant differences in total object exploration time between vehicle- or SB-399885-treated mice in either genotype. Together, these results suggest that SB-399885 significantly improved performance of NTG mice during the metric task and this

improved performance was unlikely due a reduction in anxiety or increased time spent exploring the objects.

SB-399885 Has No Effect on Seizure Threshold in J20 or NTG Mice

Next, we tested the effects of 5-HT_6 receptor antagonists on seizure threshold in both J20 and NTG mice (n = 8 per treatment group). First, we generated CC curves in J20 and NTG mice in the minimal clonic seizure threshold test using the staircase method. Results revealed that J20 mice had a significantly lower seizure threshold than NTG mice (Figure 3.4A, $p < 0.05$, Probit analysis). However, SB-399885 (30 mg / kg i.p.,) had no significant effect on seizure threshold in J20 or NTG mice when administered 30 min before testing. Additionally, two-way ANOVA of the average CC_{50} revealed a significant effect of genotype but no significant effect of drug or interaction (Figure 3.4B, $F_{(1, 28)} = 65.28$; $p < 0.001$). These results suggest that J20 mice have a lower seizure threshold compared to their NTG littermates, and that acute treatment with the 5-HT_6 receptor antagonist SB-399885 (30 mg / kg, i.p.) 30 min prior to seizure testing did not significantly affect seizure threshold in either genotype.

5-HT_6 Receptor Antagonists Have Drug- and Strain-Dependent Effects on Seizures in Mice

In addition to investigating the effects of 5-HT_6 receptor blockade on cognition and seizures in a transgenic model of AD, we also wanted to test the effects of 2 5-HT_6 receptor antagonists in 2 stains of naïve and otherwise healthy mice. Male C57BL/6 and

CF1 mice were administered SB-399885 (10 mg / kg, i.p.), SB-271046 (10 mg / kg, i.p.), or vehicle and 30 min later were stimulated at their CC_{50}. Surprisingly, all 16 C57BL/6 mice that received SB-399885 had seizures (Figure 3.5A, number of mice seized / number of mice tested: Vehicle: 11/18; SB-399885: 16/16, $p < 0.01$, Fisher's exact test). However, SB-399885 had no significant effect on seizure threshold in CF1 mice (Figure 3.5B, $p > 0.05$, Fisher's exact test). Additionally, the 5-HT_6 receptor antagonist SB-271046 had no significant effect on seizure threshold in C57BL/6 or CF1 mice (Figure 3.5C, D, $p > 0.05$, Fisher's exact test). Together, these results suggest that 5-HT_6 receptor antagonists have strain- and ligand-dependent effects on seizure threshold in mice. See Table 3.1 for a summary of the effects of 5-HT_6 receptor antagonists on seizure threshold obtained in this study.

Discussion

The present study tested the effects of 5-HT_6 receptor antagonists on cognition and seizures in a transgenic model of AD, and also explored their effects on seizures in naïve C57BL/6 and CF1 mice. Initial results in the metric task suggested that vehicle-treated NTG mice can successfully perform during the metric task, whereas vehicle-treated J20 mice may be impaired. However, in the following experiments involving 2 cohorts, both vehicle-treated NTG and J20 mice exhibited poor performances, and the 5-HT_6 receptor antagonist SB-399885 significantly improved performance in NTG mice but not in J20 mice. Additionally, SB-399885 also attenuated total distance traveled and time spent in the center in both genotypes, but had no effect on number of center crossings. Results from minimal clonic seizure threshold testing revealed that J20 mice

had a significantly lower seizure threshold compared to their NTG littermates, and SB-399885 had no effect on seizure threshold in either group. Additionally, SB-399885 lowered minimal clonic seizure threshold in C57BL/6 mice but had no effect in CF1 mice, whereas another 5-HT_6 receptor antagonist SB-271046 had no effect on seizure threshold in either strain. In summary, 5-HT_6 receptor blockade improved spatial pattern processing in NTG mice during the metric task but not in AD mice, and also had strain- and ligand-dependent proconvulsant effects in C57BL/6 and CF1 mice.

The significance of this study stems from the idea that anywhere from 1.5–64% of AD patients experience seizure (Friedman et al., 2012). Thus, new treatments for AD need to be both procognitive and anticonvulsant. Prior studies report procognitive and anticonvulsant properties of 5-HT_6 receptor antagonists, but their effects on cognition and seizures in a transgenic animal model of AD are unreported. Despite their advancement to clinical trials, understanding the effects of 5-HT_6 receptor antagonists on cognition in animal models of AD would justify laborious attempts to reverse engineer 5-HT_6 receptor physiology in transgenic animal models of AD, where remodeling of the serotonergic system and other neurotransmitter systems may alter their effects from those in otherwise healthy animals. Moreover, understanding the effects of 5-HT_6 receptor antagonists on seizures in AD models is critical because they may produce various dose-, time-, strain-dependent effects on seizures, which may also vary depending on seizure type. Thus, results from such investigations may inform their effects on seizures in humans and thus guide their clinical use.

The primary limitation in the present cognition study is NTG mice in the first cohort successfully performed during the metric task (Figure 3.1), but neither cohort

successfully performed during subsequent testing (Figure 3.2). It is unlikely that this irreproducibility is due to natural aging-related impairment in NTG mice, since the first test in Figure 3.1 was conducted between 1 and 2 weeks before the second test in Figure 3.2A, and the second cohort was tested at a similar age. Although NTG mice did initially exhibit a successful performance, the metric task was initially designed for rats (Goodrich-Hunsaker et al., 2008) and may be a difficult task for mice to consistently perform. Moreover, object exploration times could have potentially been increased by using a smaller arena (i.e., 20 X 20 cm) and objects that are incapable of being mounted by mice, which may have improved learning and produced more consistent performances by NTG mice in the metric task (Dr. Marco Bortolotto, personal communication).

Nevertheless, treatment with a 5-HT_6 receptor antagonist did significantly improve the average RID of NTG mice. This improvement is not surprising, since several studies have reported that acute systemic treatment with a 5-HT_6 receptor antagonist improved cognitive function in naïve and otherwise healthy rodents (Fone, 2008). Interestingly, both genotypes of 5-HT_6 receptor antagonist-treated mice traveled significantly less distances and spent less time in the center of the arena. Distance traveled and time spent in the center during open field and natural exploratory tasks in rodents can be used as surrogate measures of anxiety (Prut and Belzung, 2003), such that reductions in these measures are thought to be indicative of increased anxiety. Anxiogenic effects of SB-399885 is surprising since several other studies have reported anxiolytic effects of 5-HT_6 receptor antagonists. For example, acute treatment with the 5-HT_6 receptor antagonists SB-399885 or SB-258585 increased the number of open arm entries and time spent in the open arms in the elevated plus maze (EPM), and also dose-

dependently increased the number of shocks mice would withstand in the Vogel conflict drinking test, which mirrored the anxiolytic-like effects of diazepam (DZP) (Wesolowska and Nikiforuk, 2007; Wesolowska, 2008). Similarly to other anxiolytic drugs (Prut and Belzung, 2003), 5-HT_6 receptor blockade was shown to attenuate locomotion and exploratory behavior in the open field test. However, in contrast to the aforementioned anxiolytic effects of 5-HT_6 receptor blockade, others have reported anxiogenic effects following chronic treatment with a 5-HT_6 receptor-directed antisense oligonucleotide in the EPM as well as a social interaction test (Hamon et al., 1999; Otano et al., 1999). Further adding to this controversy, studies have reported anxiolytic effects of 5-HT_6 receptor *agonists* in rats during EPM testing (Carr et al., 2011). Thus, the effects of 5-HT_6 receptor activity on anxiety are complex and remain unclear. Results in our study – which show a reduction in distance traveled and time spent in the center – support the idea that SB-399885 treatment may have had anxiogenic effects. Although increased anxiety is generally thought to impair cognitive function, it is possible that SB-39985 simultaneously produced anxiogenic effects that did not interfere with its procognitive effects, and are thus mutually exclusive phenomena. It is also possible that the reduction in distance traveled and center crossings were due to sedative effects of 5-HT_6 receptor antagonists, which may have attenuated distance traveled and time spent in the center, as well as anxiety, and thus contributed to improve spatial pattern processing. Lastly, it is unlikely that the reduction in distance traveled in SB-399885-treated animals is due to increased immobility time dedicated towards object exploration (and hence, learning), since total object exploration time was reduced in SB-399885-treated animals (Figure 3.3D). Thus, results obtained in this study suggest that improved performance in SB-

399885-treated NTG mice during the metric task are possibly attributable to sedative effects of SB-399885, but not increased time spent exploring the objects, and thus may have actually improved spatial pattern processing as indicated by increased RID's in NTG mice. Nevertheless, additional studies are needed to assess the effects of 5-HT_6 receptor antagonists on spatial pattern processing, anxiety, and sedation in NTG and J20 mice.

Testing the effects of 5-HT_6 receptor antagonists on minimal clonic seizure threshold in AD and naïve mice also revealed several interesting results. For example, seizure threshold in vehicle-treated J20 mice was significantly reduced compared to vehicle-treated NTG mice (Figure 3.4). This result is not surprising since others have reported spontaneous nonconvulsive seizures as well as increased susceptibility to pentylenetetrazol- (PTZ) induced seizures in J20 mice and other transgenic models of AD (Del Vecchio et al., 2004; Palop et al., 2007). Although 5-HT_6 receptor antagonists are thought to attenuate inhibitory synaptic transmission, an effect that is generally considered proconvulsant, others studies have reported their, albeit paradoxical, anticonvulsant effects (Routledge et al., 2000; Hirst et al., 2006; Wang et al., 2015). However, we did not observe any effect of SB-399885 on seizure threshold in J20 mice.

Several factors may have contributed to a lack of effect on seizure threshold in J20 mice. For example, 5-HT_6 receptor antagonists have shown anticonvulsant effects in the MES test at 10 mg / kg in rats (Routledge et al., 2000; Hirst et al., 2006), whereas all seizure threshold testing in the present study was conducted in mice. Therefore, species differences may have contributed a lack of effect observed on seizures in J20 and NTG mice in the present study. Another possibility is the dose tested: Others have reported

inverted U-shaped concentration-response curves for a 5-HT_6 receptor ligand *in vitro* (West et al., 2009). Thus, it is possible that we did not observe an effect of SB-399885 on seizure threshold in J20 or NTG mice because 30 mg / kg was too high on the dose-response curve to impact seizure thresholds. A lack of effect on seizure threshold in J20 and NTG mice is particularly interesting considering that SB-399885 was proconvulsant at 10 mg / kg in naïve C57BL/6 mice, but had no effect in CF1 mice. Although J20 and NTG mice were backcrossed to a C57BL/6 background at least 12 times, they were originated on a partial DBA/2 mouse background (Mucke et al., 2000). Thus, it is possible that slight differences in genotype or dosing contributed to the discrepancy between the proconvulsant effects observed in C57BL/6 mice, as well as the lack of effect on seizures threshold in J20, NTG, and CF1 mice.

In addition to dose- and strain-dependent effects, we also observed ligand-dependent effects of 5-HT_6 receptor antagonists. SB-399885 had proconvulsant effects in C57BL/6 mice, whereas SB-271046 had no effect on seizure threshold in C57BL/6 or CF1 mice. This discrepancy may be due to pharmacokinetic differences between the 2 ligands. For instance, SB-399885 has been shown to exhibit a superior pharmacokinetic profile over SB-271046, with a 3-fold increase in brain penetrance, and a superior pharmacokinetic-pharmacodynamic relationship (Routledge et al., 2000; Hirst et al., 2003). The ligand-dependent effects are unlikely to be due to differences in binding affinity, since both SB-399885 and SB-271046 are high-affinity ligands with 200-fold and 50-fold selectivity, respectively, for 5-HT_6 receptors over other neurotransmitter receptor, ion channels, and enzymes. Thus, the lack of effect we observed when testing SB-271046 on minimal clonic seizure threshold may have been due to poor CNS

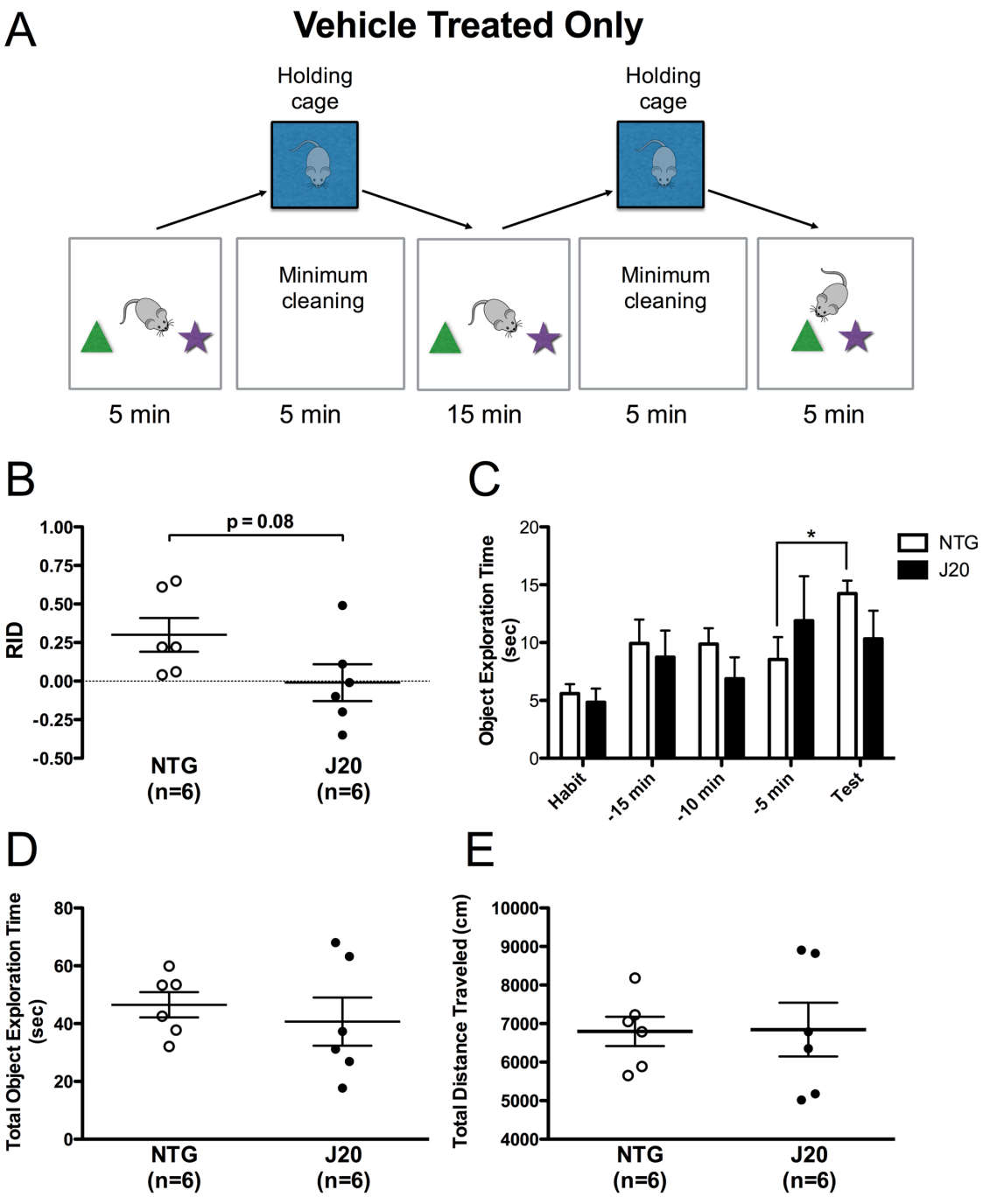
penetrance.

It is also possible that the strain- and ligand-dependent effects we observed are a false-positive. SB-399885's proconvulsant effects on seizure threshold was only tested in 1 cohort of C57BL/6 mice and thus need to be repeated and confirmed in a second cohort of animals. Furthermore, all the data obtained in this study were minimally varied with respect to dose and time-point and deserve more comprehensive testing. Future studies should test 5-HT_6 receptor antagonists for their dose-, time-, strain-, and species-dependent effects on seizures, and ultimately, their effects in multiple seizure models. The latter point is of critical importance since different seizure tests recruit different brain regions (Barton et al., 2001; Eells et al., 2004). Thus, 5-HT_6 receptor antagonists may exhibit various effects on seizures, or lack thereof, depending on seizure type. Moreover, testing each of these parameters in J20 mice and other transgenic animal models of AD is critical because 5-HT_6 receptor antagonists are currently being evaluated for their procognitive effects in humans with AD; thus, their effects on seizures, beneficial or deleterious, are unknown, and may go unnoticed in patients. Thorough evaluation of the convulsant properties of 5-HT_6 receptor antagonists in rodents would inform and perhaps advise future clinical use in patients. Lastly, 5-HT_6 receptor *agonists* have been shown to promote inhibitory synaptic transmission, and reports of their effects on seizures, to date, are lacking. Thus, future studies should consider testing the effects of 5-HT_6 receptor agonists on seizures in rodents.

In conclusion, the present study began to explore the effects of 5-HT_6 receptor antagonists on cognitive function and seizures in a transgenic mouse model of AD, as well as their effects on seizures in 2 stains of WT mice. SB-399885 did not improve

cognitive function or affect seizures in J20 mice, and did exhibit a proconvulsant profile in C57BL/6 mice. Nevertheless, these results are informative because they highlight the critical need to further evaluate the effects of 5-HT_6 receptor antagonists on seizures, and raise clinical awareness that may inform their use in humans.

Figure 3.1. Pilot study indicates J20 mice may exhibit spatial pattern processing deficits in the metric task. A) Schematic illustrating the metric task. NTG and J20 mice were treated with methyl cellulose (vehicle) 30 min prior to testing. No drug was tested in this preliminary study. B) Mean recognition index (RID) ± SEM with scatter plots for NTG and J20 mice (n = 6 per genotype). RIDs in J20 mice were not significantly different than RIDs in NTG mice, however, the results were trending towards an impaired performance in J20 mice (p = 0.08, unpaired Student's t test). C) Mean object exploration time ± SEM by epoch. NTG mice spent significantly more time exploring the objects in the test phase compared to the last 5 min of acquisition ($p < 0.05$, paired Student's t test), where J20 mice did not ($p > 0.05$). D) Mean total object exploration times ± SEM with scatter plots. NTG and J20 mice exhibited no significant differences in total object exploration times ($p > 0.05$, unpaired Student's t test). E) Mean total distance traveled ± SEM with scatter plots. No differences between J20 or NTG mice were detected ($p > 0.05$, unpaired Student's t test).


A
Vehicle Treated Only
Holding cage
Holding cage
Minimum cleaning
Minimum cleaning
5 min
5 min
15 min
5 min
5 min
B
p = 0.08
RID
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
NTG (n=6)
J20 (n=6)
C
Object Exploration Time (sec)
20
15
10
5
0
*
NTG
J20
Habit
-15 min
-10 min
-5 min
Test
D
Total Object Exploration Time (sec)
80
60
40
20
0
NTG (n=6)
J20 (n=6)
E
Total Distance Traveled (cm)
10000
9000
8000
7000
6000
5000
4000
NTG (n=6)
J20 (n=6)

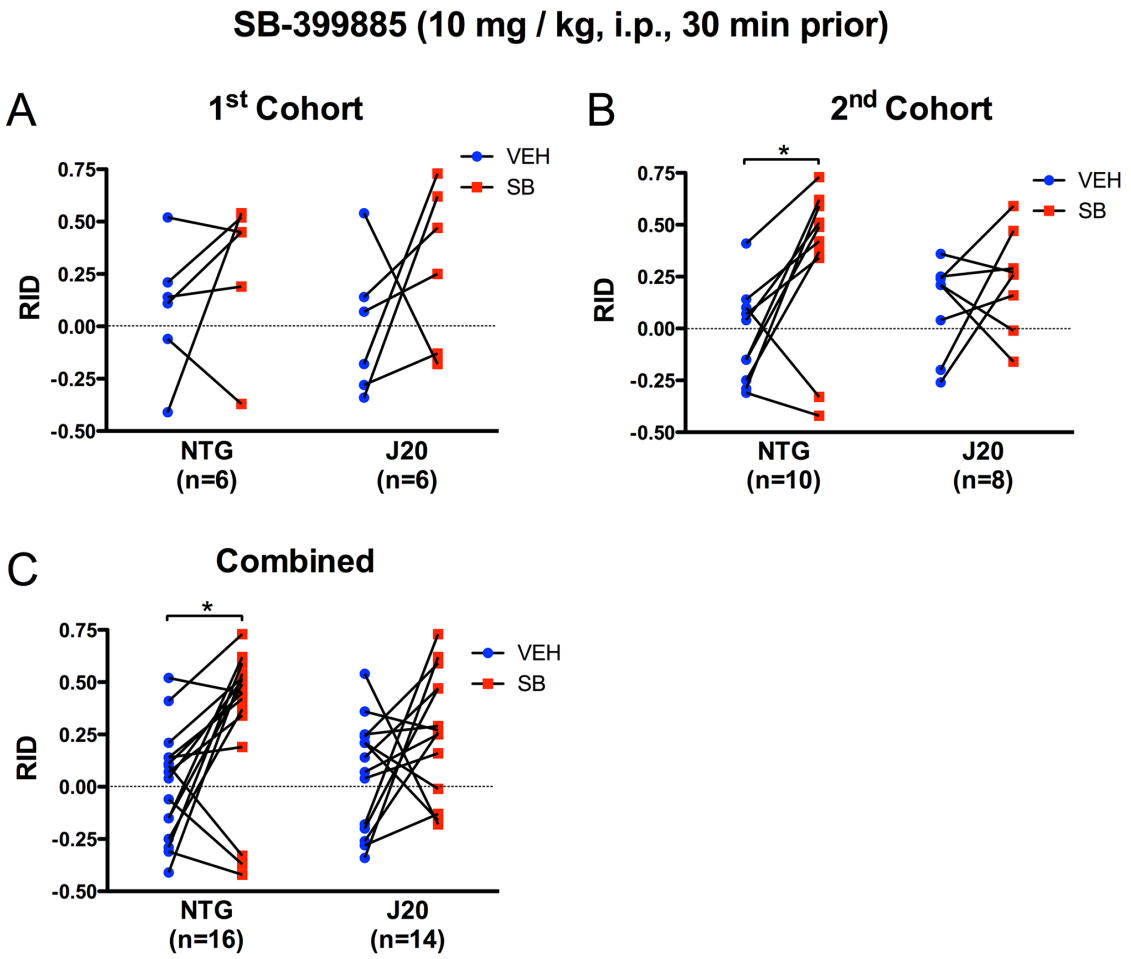


Figure 3.2. SB-399885 improved the performance of NTG but not J20 mice in the metric task. A) Paired scatterplot of recognition index (RID) for vehicle (blue) and SB-399885-treated (red) NTG and J20 mice (n = 6 per genotype and treatment group). The same cohort tested in Figure 3.1 was retested 1 week later in the metric task; only this time, mice were treated with methyl cellulose (VEH, 0.5% methyl cellulose, i.p.) or SB-399885 (SB, red, 10 mg / kg, i.p.) 30 min prior to first 5 min epoch using a crossover experimental design, so that each mouse was tested following treatment with VEH or SB. Acute treatment with SB-399885 did not significantly improve the average RID compared to VEH-treated NTG or J20 mice (p = 0.13, two-way repeated measures ANOVA with Bonferroni's post hoc test). B) Paired scatterplots of RIDs for a second cohort of NTG and J20 mice were also tested in a crossover experimental design (n = 10 and 8, respectively). SB significantly increased the RID of NTG mice only (* $p < 0.05$, two-way repeated measures ANOVA with Bonferroni's post hoc test). C) Paired scatterplots of RIDs for both cohorts combined. SB-treated NTG mice performed significantly better than vehicle treated NTG mice (* $p < 0.05$, two-way repeated measures ANOVA with Bonferroni's post hoc test). No significant improvement was detected in J20 mice.

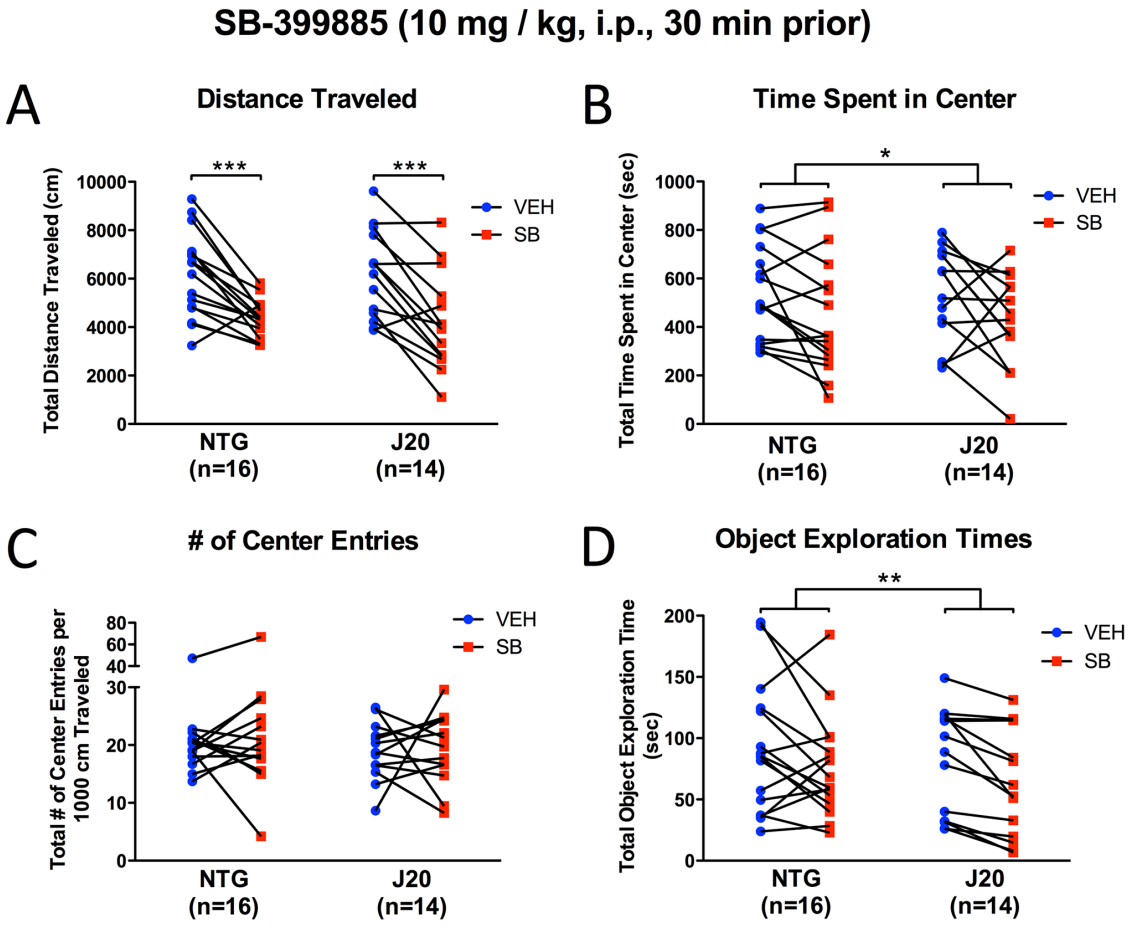


Figure 3.3. SB-399885 attenuated total distance traveled, total time spent in center, and total object exploration time in NTG and J20 mice during the metric task. A) Paired scatterplot of total distance traveled in vehicle-treated (VEH, blue) and SB-399885-treated (SB, red, 10 mg / kg, i.p., 30 min) in NTG and J20 mice (n = 16 and 14, respectively). SB-treatment significantly attenuated distance traveled in both NTG and J20 drug-treated mice compared to vehicle-treated and uninjected NTG and J20 mice, respectively (*** $p < 0.001$, two-way repeated measures ANOVA with Bonferroni's post hoc test). B) Paired scatterplot of total time spent in the center zone for VEH- and SB-treated NTG and J20 mice. A significant effect of drug on total time spent in the center was observed (* $p < 0.05$, two-way repeated measures ANOVA). However, Bonferroni's multiple comparisons revealed no significant reduction in total times spent in the center in either genotype C) Paired scatterplot of total number of center entries normalized to distance traveled. No significant effects were detected ($p > 0.05$, two-way repeated measures ANOVA). D) Paired scatterplot of total object exploration time. A significant effect of drug was observed (** $p < 0.01$, two-way repeated measures ANOVA). Bonferroni's multiple comparisons revealed no significant effect in either genotype.

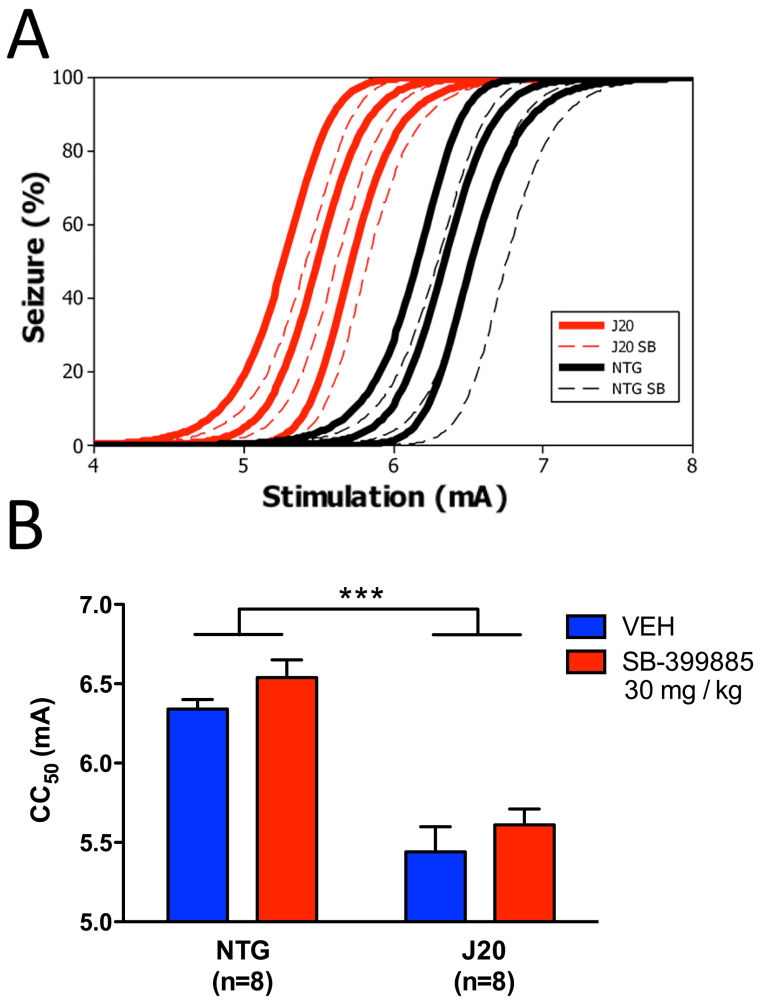


Figure 3.4. J20 mice have a lower seizure threshold in the minimal clonic seizure test that is unaffected by SB-399885. A) Convulsive current (CC) curves for J20 (red) and NTG mice (black) treated with vehicle (VEH, solid lines) or SB-399885 (SB, dashed lines, 30 mg / kg, i.p., 30 min) in the minimal clonic seizure threshold test (n = 8 per treatment group). Middle line is the CC; flanked lines represent the 95% CI. J20 mice have significantly a lower seizure threshold compared to NTG mice (Probit analysis, $p < 0.05$). B) CC_{50} for VEH-treated (blue) and SB-treated (red) NTG and J20 mice. J20 mice had significantly lower CC_{50} compared to NTG mice, but SB had no effect on seizure threshold in either group (*** $p < 0.001$, two-way ANOVA with Bonferroni's post hoc test). This experiment was conducted by Dr. Peter J. West.

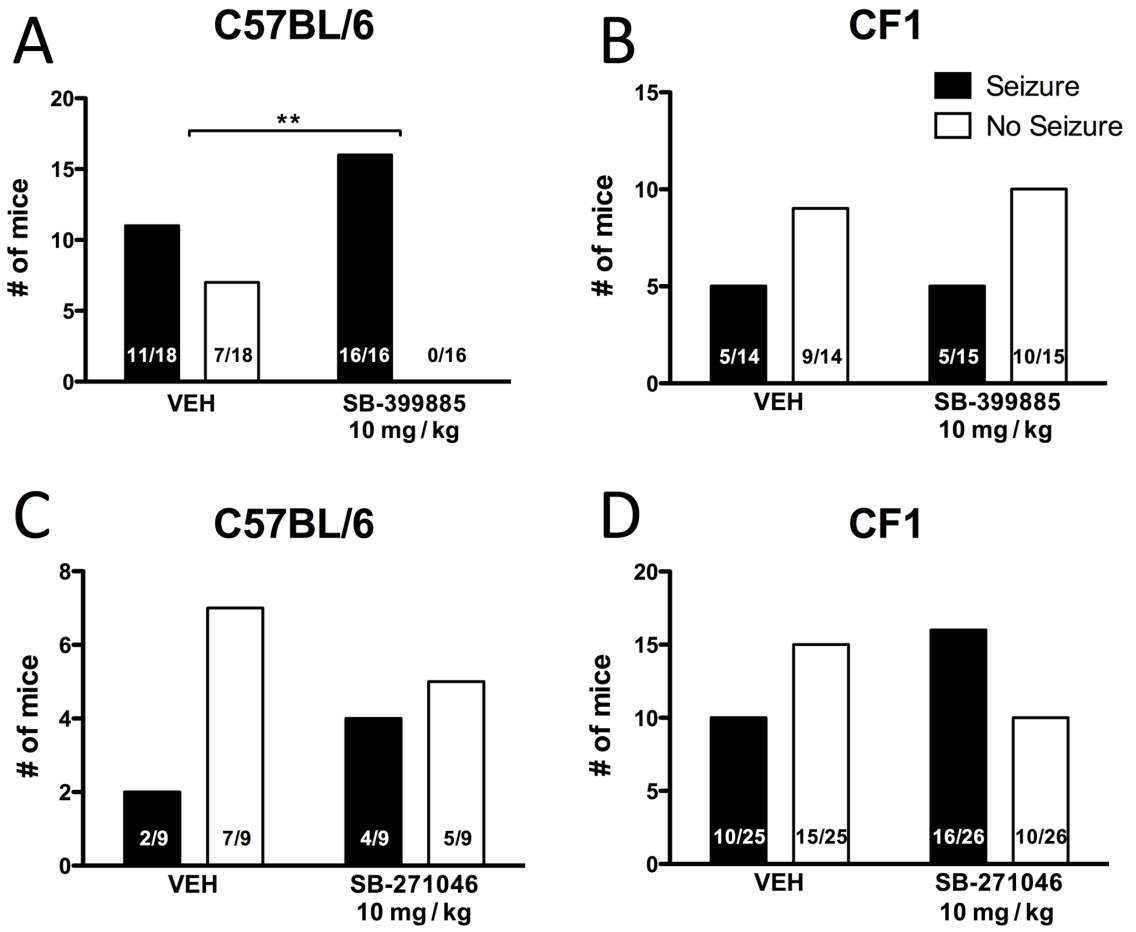


Figure 3.5. 5-HT_6 receptor antagonists have strain- and ligand-dependent effects on seizure threshold in mice. A) Number of C57BL/6 mice to exhibit seizures (black) versus the number of mice that did not have seizures (white) during the minimal clonic seizure test when treated with vehicle (VEH) or SB-399885 (10 mg / kg, i.p., 30 min). SB-399885 caused a greater number of C57BL/6 mice to have seizures compared to VEH-treated mice (n = # of mice seized or not seized and the total # of mice tested, ** $p <$ 0.01, Fisher's exact test). B) SB-39985 had no effect on seizure threshold in CF1 mice (p > 0.05, Fisher's exact test). C) The 5-HT_6 receptor antagonist SB-271046 (10 mg / kg, i.p., 30 min) did not alter seizure threshold in C57BL/6 mice or, D) CF1 mice (p > 0.05 for C and D, Fisher's exact test).

Table 3.1

Summary of 5-HT_6 receptor antagonist effects on minimal clonic seizure threshold

Strain	SB-399885	SB-271046
J20	No effect	Not tested
NTG	No effect	Not tested
C57BL/6	Proconvulsant	No effect
CF1	No effect	No effect

References

Alzheimer's Association (2015) 2015 Alzheimer's disease facts and figures. Alzheimers Dement 11:332-384.

Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, Yassa MA, Bassett SS, Shelton AL, Gallagher M (2012) Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron 74:467-474.

Barton ME, Klein BD, Wolf HH, White HS (2001) Pharmacological characterization of the 6 Hz psychomotor seizure model of partial epilepsy. Epilepsy Res 47:217-227.

Carr GV, Schechter LE, Lucki I (2011) Antidepressant and anxiolytic effects of selective 5-HT6 receptor agonists in rats. Psychopharmacology (Berl) 213:499-507.

Codony X, Vela JM, Ramirez MJ (2011) 5-HT(6) receptor and cognition. Curr Opin Pharmacol 11:94-100.

Cumbo E, Ligori LD (2010) Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. Epilepsy Behav 17:461-466.

Da Silva Costa V, Duchatelle P, Boulouard M, Dauphin F (2009) Selective 5-HT6 receptor blockade improves spatial recognition memory and reverses age-related deficits in spatial recognition memory in the mouse. Neuropsychopharmacology 34:488-500.

Dawson LA, Nguyen HQ, Li P (2001) The 5-HT(6) receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. Neuropsychopharmacology 25:662-668.

Del Vecchio RA, Gold LH, Novick SJ, Wong G, Hyde LA (2004) Increased seizure threshold and severity in young transgenic CRND8 mice. Neurosci Lett 367:164-167.

Eells JB, Clough RW, Browning RA, Jobe PC (2004) Comparative fos immunoreactivity in the brain after forebrain, brainstem, or combined seizures induced by electroshock, pentylenetetrazol, focally induced and audiogenic seizures in rats. Neuroscience 123:279-292.

Ennaceur A (2010) One-trial object recognition in rats and mice: methodological and theoretical issues. Behav Brain Res 215:244-254.

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res 31:47-59.

Foley AG, Murphy KJ, Hirst WD, Gallagher HC, Hagan JJ, Upton N, Walsh FS, Regan CM (2004) The 5-HT(6) receptor antagonist SB-271046 reverses scopolamine-disrupted consolidation of a passive avoidance task and ameliorates spatial task deficits in aged rats. Neuropsychopharmacology 29:93-100.

Fone KC (2008) An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. Neuropharmacology 55:1015-1022.

Friedman D, Honig LS, Scarmeas N (2012) Seizures and epilepsy in Alzheimer's disease. CNS Neurosci Ther 18:285-294.

Garcia-Alloza M, Hirst WD, Chen CP, Lasheras B, Francis PT, Ramirez MJ (2004) Differential involvement of 5-HT(1B/1D) and 5-HT6 receptors in cognitive and non-cognitive symptoms in Alzheimer's disease. Neuropsychopharmacology 29:410-416.

Gaugler JE, Yu F, Krichbaum K, Wyman JF (2009) Predictors of nursing home admission for persons with dementia. Med Care 47:191-198.

Gold CA, Budson AE (2008) Memory loss in Alzheimer's disease: implications for development of therapeutics. Expert Rev Neurother 8:1879-1891.

Goodrich-Hunsaker NJ, Hunsaker MR, Kesner RP (2008) The interactions and dissociations of the dorsal hippocampus subregions: how the dentate gyrus, CA3, and CA1 process spatial information. Behav Neurosci 122:16-26.

Hamed SA (2009) The aspects and mechanisms of cognitive alterations in epilepsy: the role of antiepileptic medications. CNS Neurosci Ther 15:134-156.

Hamon M, Doucet E, Lefevre K, Miquel MC, Lanfumey L, Insausti R, Frechilla D, Del Rio J, Verge D (1999) Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT6 receptors. Neuropsychopharmacology 21:68S-76S.

Helmstaedter C, Mihov Y, Toliat MR, Thiele H, Nuernberg P, Schoch S, Surges R, Elger CE, Kunz WS, Hurlemann R (2013) Genetic variation in dopaminergic activity is associated with the risk for psychiatric side effects of levetiracetam. Epilepsia 54:36-44.

Hirst WD, Abrahamsen B, Blaney FE, Calver AR, Aloj L, Price GW, Medhurst AD (2003) Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. Mol Pharmacol 64:1295-1308.

Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM, Riley G, Smith DR, Bartlett S, Heidbreder CA, Atkins AR, Lacroix LP, Dawson LA, Foley AG, Regan CM, Upton N (2006) SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol 553:109-119.

Kendall I, Slotten HA, Codony X, Burgueno J, Pauwels PJ, Vela JM, Fone KC (2011) E-6801, a 5-HT6 receptor agonist, improves recognition memory by combined modulation of cholinergic and glutamatergic neurotransmission in the rat.

Psychopharmacology (Berl) 213:413-430.

King MV, Sleight AJ, Woolley ML, Topham IA, Marsden CA, Fone KC (2004) 5-HT6 receptor antagonists reverse delay-dependent deficits in novel object discrimination by enhancing consolidation--an effect sensitive to NMDA receptor antagonism. Neuropharmacology 47:195-204.

Kleen JK, Scott RC, Holmes GL, Roberts DW, Rundle MM, Testorf M, Lenck-Santini PP, Jobst BC (2013) Hippocampal interictal epileptiform activity disrupts cognition in humans. Neurology 81:18-24.

Kumlien E, Lundberg PO (2010) Seizure risk associated with neuroactive drugs: data from the WHO adverse drug reactions database. Seizure 19:69-73.

Lee I, Hunsaker MR, Kesner RP (2005) The role of hippocampal subregions in detecting spatial novelty. Behav Neurosci 119:145-153.

Lee S, Sziklas V, Andermann F, Farnham S, Risse G, Gustafson M, Gates J, Penovich P, Al-Asmi A, Dubeau F, Jones-Gotman M (2003) The effects of adjunctive topiramate on cognitive function in patients with epilepsy. Epilepsia 44:339-347.

Loscher W (1998) Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy. Prog Neurobiol 54:721-741.

Maher-Edwards G, Dixon R, Hunter J, Gold M, Hopton G, Jacobs G, Hunter J, Williams P (2011) SB-742457 and donepezil in Alzheimer disease: a randomized, placebo-controlled study. Int J Geriatr Psychiatry 26:536-544.

Mares P, Mikulecka A (2009) Different effects of two N-methyl-D-aspartate receptor antagonists on seizures, spontaneous behavior, and motor performance in immature rats. Epilepsy Behav 14:32-39.

McAreavey MJ, Ballinger BR, Fenton GW (1992) Epileptic seizures in elderly patients with dementia. Epilepsia 33:657-660.

Meador KJ (2002) Cognitive outcomes and predictive factors in epilepsy. Neurology 58:S21-26.

Meador KJ, Loring DW, Abney OL, Allen ME, Moore EE, Zamrini EY, King DW (1993) Effects of carbamazepine and phenytoin on EEG and memory in healthy adults. Epilepsia 34:153-157.

Meador KJ, Loring DW, Moore EE, Thompson WO, Nichols ME, Oberzan RE, Durkin MW, Gallagher BB, King DW (1995) Comparative cognitive effects of phenobarbital, phenytoin, and valproate in healthy adults. Neurology 45:1494-1499.

Meador KJ, Loring DW, Vahle VJ, Ray PG, Werz MA, Fessler AJ, Ogrocki P, Schoenberg MR, Miller JM, Kustra RP (2005) Cognitive and behavioral effects of

lamotrigine and topiramate in healthy volunteers. Neurology 64:2108-2114.

Mitchell ES, Hoplight BJ, Lear SP, Neumaier JF (2006) BGC20-761, a novel tryptamine analog, enhances memory consolidation and reverses scopolamine-induced memory deficit in social and visuospatial memory tasks through a 5-HT6 receptor-mediated mechanism. Neuropharmacology 50:412-420.

Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L (2000) High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci 20:4050-4058.

Nygaard HB (2013) Current and emerging therapies for Alzheimer's disease. Clin Ther 35:1480-1489.

Otano A, Frechilla D, Cobreros A, Cruz-Orive LM, Insausti A, Insausti R, Hamon M, Del Rio J (1999) Anxiogenic-like effects and reduced stereological counting of immunolabelled 5-hydroxytryptamine6 receptors in rat nucleus accumbens by antisense oligonucleotides. Neuroscience 92:1001-1009.

Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55:697-711.

Peltz G, Pacific DM, Noviasky JA, Shatla A, Mehalic T (2005) Seizures associated with memantine use. Am J Health Syst Pharm 62:420-421.

Prut L, Belzung C (2003) The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 463:3-33.

Raina P, Santaguida P, Ismaila A, Patterson C, Cowan D, Levine M, Booker L, Oremus M (2008) Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. Ann Intern Med 148:379-397.

Ramirez MJ, Lai MK, Tordera RM, Francis PT (2014) Serotonergic therapies for cognitive symptoms in Alzheimer's disease: rationale and current status. Drugs 74:729-736.

Rogers DC, Hagan JJ (2001) 5-HT6 receptor antagonists enhance retention of a water maze task in the rat. Psychopharmacology (Berl) 158:114-119.

Routledge C, Bromidge SM, Moss SF, Price GW, Hirst W, Newman H, Riley G, Gager T, Stean T, Upton N, Clarke SE, Brown AM, Middlemiss DN (2000) Characterization of SB-271046: a potent, selective and orally active 5-HT(6) receptor antagonist. Br J Pharmacol 130:1606-1612.

Salinsky MC, Storzbach D, Spencer DC, Oken BS, Landry T, Dodrill CB (2005) Effects of topiramate and gabapentin on cognitive abilities in healthy volunteers. Neurology 64:792-798.

Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu GQ, Palop JJ, Mucke L (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc Natl Acad Sci U S A 109:E2895-2903.

Schechter LE, Lin Q, Smith DL, Zhang G, Shan Q, Platt B, Brandt MR, Dawson LA, Cole D, Bernotas R, Robichaud A, Rosenzweig-Lipson S, Beyer CE (2008) Neuropharmacological profile of novel and selective 5-HT6 receptor agonists: WAY-181187 and WAY-208466. Neuropsychopharmacology 33:1323-1335.

Smith GK, Kesner RP, Korenberg JR (2014) Dentate gyrus mediates cognitive function in the Ts65Dn/DnJ mouse model of down syndrome. Hippocampus 24:354-362.

Stean TO, Hirst WD, Thomas DR, Price GW, Rogers D, Riley G, Bromidge SM, Serafinowska HT, Smith DR, Bartlett S, Deeks N, Duxon M, Upton N (2002) Pharmacological profile of SB-357134: a potent, selective, brain penetrant, and orally active 5-HT(6) receptor antagonist. Pharmacol Biochem Behav 71:645-654.

Volicer L, Smith S, Volicer BJ (1995) Effect of seizures on progression of dementia of the Alzheimer type. Dementia 6:258-263.

Wang L, Lv Y, Deng W, Peng X, Xiao Z, Xi Z, Chen G, Wang X (2015) 5-HT6 receptor recruitment of mTOR modulates seizure activity in epilepsy. Mol Neurobiol 51:1292-1299.

Wesolowska A (2008) The anxiolytic-like effect of the selective 5-HT6 receptor antagonist SB-399885: the impact of benzodiazepine receptors. Eur J Pharmacol 580:355-360.

Wesolowska A, Nikiforuk A (2007) Effects of the brain-penetrant and selective 5-HT6 receptor antagonist SB-399885 in animal models of anxiety and depression. Neuropharmacology 52:1274-1283.

West PJ, Marcy VR, Marino MJ, Schaffhauser H (2009) Activation of the 5-HT(6) receptor attenuates long-term potentiation and facilitates GABAergic neurotransmission in rat hippocampus. Neuroscience 164:692-701.

Wilkinson D, Windfeld K, Colding-Jorgensen E (2014) Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Neurol 13:1092-1099.

Wimo A, Jonsson L, Bond J, Prince M, Winblad B, Alzheimer Disease I (2013) The worldwide economic impact of dementia 2010. Alzheimers Dement 9:1-11 e13.

Woolley ML, Bentley JC, Sleight AJ, Marsden CA, Fone KC (2001) A role for 5-ht6 receptors in retention of spatial learning in the Morris water maze. Neuropharmacology 41:210-219.

CHAPTER 4

CORNEAL KINDLED C57BL/6 MICE EXHIBIT DG-ASSOCIATED MEMORY DEFICITS AND HYPEREXCITABILITY IN THE ABSENCE OF OVERT HIPPOCAMPAL NEURON LOSS[1]

Abstract

Memory deficits have a significant impact on quality of life of the patient with epilepsy and currently no effective treatments exist to mitigate this comorbidity. Although epilepsy patients exhibit varying degrees of hippocampal cell death and hippocampal sclerosis (HS), not all patients with memory impairments exhibit severe cell loss, and HS is not always associated with memory deficits. Thus, more subtle changes in hippocampal physiology may underlie memory dysfunction in some epilepsy patients. Animal models of epilepsy or epileptic processes exhibiting memory deficits in the absence of cell loss could facilitate novel therapy discovery. Following corneal kindling, a noninvasive method of kindling, C57BL/6 mice exhibited deficits during a DG-

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associated spatial memory test – the metric task. Quantitative immunohistochemical analysis revealed hippocampal astrogliosis in the absence of overt neuron loss. Additionally, using *in vitro* brain slice electrophysiology, several changes in synaptic transmission were detected suggestive of hyperexcitability in dentate granule cells (DGCs): Input-output I/O curves revealed DGCs have an increased postsynaptic response and reduced threshold to population spikes during perforant path (pp) stimulation, and voltage-clamp recordings from DGCs revealed increased membrane resistance and amplitude of spontaneous excitatory events. We also observed attenuated long-term potentiation (LTP) at the pp-DGC synapse. These changes, observed in the absence of alterations to paired-pulse ratios (PPRs) and inhibitory synaptic transmission, suggest that DGCs from corneal kindled mice are hyperexcitable and have long-term synaptic plasticity deficits. These changes may contribute to impaired spatial pattern processing in corneal kindled mice and may represent a therapeutic target to treat seizure-related memory deficits.

Introduction

Epilepsy is a neurological disorder that affects 1 in 26 individuals worldwide (Hesdorffer et al., 2011). Besides spontaneous recurrent seizures, patients with epilepsy frequently experience cognitive comorbidities. For example, approximately 1 in 4 individuals with temporal lobe epilepsy exhibit cognitive impairment during neuropsychological evaluation, most notably on memory tests (Hermann et al., 2006). Despite the need, there are currently no treatments for memory deficits experienced by patients with epilepsy that can exert a significant impact on the epilepsy patient's quality

of life (Giovagnoli and Avanzini, 2000; Hrabok et al., 2013). The discovery of treatments for this constellation of debilitating cognitive symptoms would be greatly facilitated by an improved understanding of the underlying pathophysiology contributing to these symptoms.

Patients with epilepsy exhibit varying degrees of cell death and hippocampal sclerosis (HS) (de Lanerolle et al., 2012), and similar neuronal damage can be recapitulated in a number of animal models (Friedman et al., 1994; Covolan and Mello, 2000; Rao et al., 2006; Groticke et al., 2008; Chauviere et al., 2009). However, not all epilepsy patients demonstrating memory impairment have severe hippocampal damage (Aikia et al., 1995; Lah et al., 2014), and hippocampal cell loss with or without HS is not always associated with memory impairment (Castro et al., 2013; Schmidt et al., 2015). Moreover, associations between hippocampal volume loss and performance on memory tests are highly variable (Aikia et al., 2001). These observations suggest, at least in some epilepsy patients, more subtle changes in hippocampal physiology may underlie memory dysfunction. Thus, a better understanding of these physiological changes is needed, and animal models of epileptic processes that exhibit memory dysfunction without cell loss or HS would be particularly useful.

Rodent kindling models of epilepsy that employ direct electrical stimulation of limbic structures exhibit minimal neuronal loss (Tooyama et al., 2002; Brandt et al., 2004) and spatial memory impairment (Hannesson and Corcoran, 2000) but suffer from the labor intensive nature of invasive electrode implantation surgeries. However, the corneal kindling model of seizures in mice, which has been validated as a rapid screening model of focal seizures that secondarily generalize in humans, has become a useful tool

in the discovery of novel antiseizure drugs, in part, because of its noninvasive, cost-effective facile methodology (Matagne and Klitgaard, 1998; Potschka and Loscher, 1999; Rowley and White, 2010). Moreover, Loewen et al. (2016) recently reported that corneal kindled CF1 mice do not exhibit significant neuronal loss within hippocampal area CA1, but do exhibit astrogliosis and microglia activation. Although these data suggest that corneal kindled mice may provide a noninvasive model of focal seizures that secondarily generalize that lacks overt hippocampal neuron loss, it is unknown whether corneal kindling in other strains, particularly C57BL/6 mice, similarly spare hippocampal neurons. Importantly, alteration of hippocampal physiology, particularly in mice that demonstrate learning and memory impairment without hippocampal cell loss**,** may provide insight into the relationships between pathophysiological remodeling of the hippocampus in epilepsy and cognitive comorbidities.

Therefore, we tested the hypothesis that corneal kindled mice exhibit spatial pattern processing deficits in a task reliant on dentate gyrus (DG) function in the absence of cell loss. To accomplish this, we evaluated neurodegeneration in the hippocampus of corneal kindled C57BL/6 mice as well as their performance in a DG-mediated spatial pattern processing test that relies on the rodents' natural tendencies to explore changes in the distance between 2 objects – the "metric task" (Ennaceur and Delacour, 1988; Lee et al., 2005; Goodrich-Hunsaker et al., 2008; Ennaceur, 2010). Next, we examined physiological changes in the DG of corneal kindled mice that may contribute to hyperexcitability and/or memory impairment. We used *in vitro* extracellular and whole-cell patch clamp electrophysiology to evaluate changes in synaptic transmission, granule cell excitability, and short- and long-term synaptic plasticity in the DG. Our results

demonstrate that corneal kindled C57BL/6 mice exhibit DG-associated cognitive deficits associated with pathophysiological remodeling of excitatory synaptic transmission and granule cell excitability in the DG in the absence of overt cell loss.

Methods

Animals

Forty, 5- to 6-week-old male C57BL/6 mice (15–20 g, Charles River, Raleigh, NC, U.S.A.) were used in this study. The first cohort (n = 20) of mice was used for the metric task, immunofluorescence, and synaptic plasticity experiments. The second cohort (n = 20) was used for basal synaptic transmission and patch clamp electrophysiology experiments. All experiments were conducted between 3 days and 2 weeks after kindling. All mice were group housed in a light- and temperature-controlled (12 h on / 12 h off) environment and permitted access to food and water *ad libitum* throughout the study. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Utah Institutional Animal Care and Use Committee. All efforts were made to minimize the number and suffering of animals used.

Corneal Kindling

C57Bl/6 mice were corneal kindled using a protocol adapted from CF1 mice (Rowley and White, 2010). Briefly, a 0.9% saline solution containing 0.5% tetracaine hydrochloride was applied to each eye to provide local anesthesia and electrical conductivity. Two cohorts of 10 mice were stimulated twice daily (4 h apart) with corneal

stimulation of 1.5 mA (60 Hz) for 3 s. Mice were considered fully kindled when 5 consecutive stage 5 seizures were achieved according to a slightly modified Racine scale (Racine, 1972): 0 = no reaction or immobility; 1 = jaw clonus; 2 = myoclonic twitches in the forelimbs and head nodding; 3 = clonic convulsions in the forelimbs; 4 = clonic convulsions in the forelimbs with rearing and falling; and 5 = generalized clonic convulsions associated with loss of balance. Mice were stimulated daily until 5 days before being evaluated for memory deficits and stimulated once 3 days before beginning electrophysiology experiments to confirm persistence of the fully-kindled state. Additionally, 2 cohorts of control mice (n = 10 per cohort) were also brought into the kindling facility and handled but not stimulated.

Metric Task

Habituation for the metric task began 3 days after ceasing daily kindling stimulation. At the start of each day, 1 cohort of mice (n = 10 kindled, n = 10 control, 20–25 g) was acclimated to the testing room for 1 h prior to behavioral testing. Mice were then individually habituated to a square plexiglass arena (40 L x 40 W x 60 H cm) for 2 consecutive days by freely exploring the testing arena for 10 min. Animals were then placed into an empty holding cage for 5 min, and returned to the arena for an additional 5 min of exploration. During habituation, the arena contained 2 objects in separate locations that were not used during testing and were not moved during habituation. Distinct visual spatial cues were always located 15 cm from each side of the arena. On day 3, testing was conducted in 3 phases: during phase 1 (acquisition), mice were individually allowed to explore the arena that contained 2 dissimilar objects placed 30 cm

apart for 15 min. In phase 2 (delay), the animal was removed from the arena and placed in a holding cage for 5 min, while the arena underwent a minimal cleaning protocol to wipe up any feces and exchange the objects for a duplicate precleaned pair, with the objects now placed 8 cm apart. During phase 3 (testing), the mouse was placed back in the arena for an additional 5 min. Before each trial, the arena and 4 objects (a duplicate pair of dissimilar objects) were precleaned with a 4% HDQ solution.

During testing, animals were tracked using EthoVision software (Noldus, Leesburg, VA, U.S.A.). An observer who was "blinded" to the experimental group manually scored object exploration. Exploration was defined as investigative behavior, where the mouse was orienting its head towards the object when within 2 cm of the object, or touching or smelling the object with its nose, whiskers, or forepaws. A mouse that was climbing on the objects, or in close proximity without actively attending to the objects, was not counted as exploration. Throughout phases 1 and 3, total object exploration time, regardless of which object was being explored, was recorded in 5 min epochs, expressed in seconds by epoch, and averaged by experimental group. Performance was also expressed as a recognition index (RID):

$$\mathrm{RID} = \frac{(\text{object exploration})_{\text{test}} - (\text{object exploration})_{\text{acquisition 10–15 min}}}{(\text{object exploration})_{\text{test}} + (\text{object exploration})_{\text{acquisition 10–15 min}}}$$

RIDs were calculated for each mouse and averaged by treatment group.

Immunofluorescence

All immunohistochemistry was performed as reported by Loewen et al. (2016). Briefly, 4 mice (20–25g) per treatment group were anesthetized and transcardially perfused with 0.1M phosphate-buffer solution (PBS) followed by 4% paraformaldehyde

(PFA). Brains were removed, postfixed in 4% PFA at 4°C for 7 hours, transferred to a 15/30% sucrose gradient for cryoprotection, and frozen on dry ice. Next, brains were coronally cut on a freezing stage microtome (Leica Microsystems, Buffalo Grove, IL, U.S.A) into 25 μm-thick sections through dorsal hippocampus and stored in 0.1M PBS at 4°C. Sections immediately caudal to the septum (3 sections/animal for each stain; 4 animals/treatment group) from corneal kindled and control animals were slide mounted and batch processed. First, sections were washed 3 times with 0.1 M PBS and blocked with 0.25% bovine serum albumin and 0.2% Triton X-100 in PBS for 1 h. Sections were then incubated with NeuN 555 MAB377A5 (1:500, EMD Millipore LLC, Billerica, MD, U.S.A.) and GFAP C9205 (1:1000, Sigma-Aldrich, St. Louis, MO, U.S.A.) for 2 h. All slides were then counterstained with DAPI D9542 (1:100, Sigma-Aldrich), rinsed in 0.1M PBS and coversliped with ProLong® Gold antifade reagent (Thermo Fisher Scientific, Grand Island, NY, U.S.A.).

Imaging and Analysis

Images were captured with a Nikon A1 confocal microscope (Nikon Instruments, Melville, NY, U.S.A.) using 20x/NA 1.0 air and 60x/NA 1.0 oil immersion objectives and analyzed utilizing NIS Software. Briefly, the left dorsal DG, hilus, and CA1 were identified using a 100 W halogen lamp and a filter set for DAPI to avoid selection bias. Once the region was selected, laser-scanning mode was used to collect images in the z-axis. A minimum of 12 z-stack optical images (1 μm thick) were imaged for each stain from triplicate sections.

Raw grey scale 16-bit images from the channel of each cell marker (NeuN and

GFAP) were utilized to perform quantification analysis. To remove bias in the analysis, an automated macro created using ImageJ software (National Institutes of Health) was utilized (Loewen et al., 2016). The total field area stained by each cell marker was measured for each optical section through the stained tissue section. These values were then averaged for each tissue section and averaged by treatment group and brain region.

Hippocampal Brain Slice Preparation

Acute hippocampal brain slices were prepared daily as described previously (West et al., 2014) from either a kindled or control mouse beginning 3 days-post kindling. Briefly, mice (20–25 g) were anesthetized with sodium pentobarbital (60 mg / kg, i.p.), and brains were rapidly removed and submerged in ice-cold (4°C) oxygenated sucrose-based artificial cerebral spinal fluid (ACSF) bubbled with 95% O_2/5% CO_2. Sucrose-based ACSF contained the following (in mM): Sucrose (200.0), KCl (3.0), Na_2PO_4 (1.4), $MgSO_4$ (3.0), $NaHCO_3$ (26.0), glucose (10.0), and $CaCl_2$ (0.5). The pH and osmolarity of the sucrose-based ACSF were adjusted to 7.30–7.35 and 290–300 mOsm, respectively, before each experiment. Next, coronal brain slices (350 μm) containing dorsal hippocampus were cut using a vibrating microtome (VT1000S, Leica Microsystems Inc.). Slices were then transferred to oxygenated standard ACSF and incubated for 2 h at room temperature prior to recording. Standard ACSF was made from the same recipe as sucrose-based ACSF, only sucrose was replaced with NaCl (126.0 mM), the $MgSO_4$ concentration lowered to 1.0 mM, and the $CaCl_2$ concentration raised to 2.5 mM.

Long-Term Potentiation of Excitatory Synaptic Transmission

Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded by transferring 8 coronal brain slices containing dorsal hippocampus from either hemisphere into the integrated brain slice chambers (IBSCs) of the Scientifica Slicemaster (Scientifica, Ukfield, East Sussex, U.K.), a high throughput semiautomatic brain slice recording system, which conducts separate but simultaneous recordings (Stopps et al., 2004). All slices were perfused with regular ACSF (2 mL/min) containing 10 μM picrotoxin (PTX) at 30°C unless noted otherwise. Twisted Nichrome-Formvar stimulating electrodes were placed in the middle-third of the molecular layer in the upper blade of the DG to stimulate the medial perforant path; recording electrodes were placed in the middle-third of the molecular layer approximately 400 – 600 μm away from the stimulating electrode. Input/output (I/O) relationships were measured by incrementally stimulating for 100 μs in 0.5 V steps until a population spike was observed or 20 V maximum stimulus intensity was reached. Data were acquired using pClamp 10 interfaced to a Digidata 1550A data acquisition board (Molecular Devices, Sunnyvale, CA, U.S.A.) at a sampling rate of 10 kHz, low-pass filtered at 1 kHz, and high-pass filtered at 3 Hz. The magnitude of the fEPSP was determined by measuring the 20–80% slope of the rising phase and expressed as an average for each stimulation strength by treatment group.

After obtaining fEPSPs and conducting I/O curves, baseline stimulation strength was set to 50% and slices received 1 stimulation pulse every 30 s. Once stable baselines were obtained for 30 min (maximum allowable fEPSP amplitudes drift ± 20%), LTP was induced via theta-burst stimulation (TBS) (pulse duration = 200 μs; intra-train frequency

= 100 Hz; intertrain frequency = 5 Hz; 4 trains of 4 pulses = 1 burst; 4 bursts with an interburst interval of 20 s). LTP induction was then followed by 60 min of single stimulation pulses every 30 s. All fEPSPs were normalized to the average slope of the last 5 fEPSPs prior to TBS.

Basal Excitatory Synaptic Transmission and Short-Term Plasticity

Eight coronal brain slices containing dorsal hippocampus from either hemisphere were moved into the IBSCs of the Scientifica Slicemaster. Twisted Nichrome-Formvar wire stimulating electrodes were placed in the middle third of the molecular layer of the upper blade of the DG to stimulate the medial perforant path; recording electrodes were placed in the middle third approximately 400 – 600 μm away from the stimulating electrode. I/O curves were conducted as described above, except stimulus intensity went to 20V regardless of the appearance of a population spike, and responses for each stimulation strength were averaged by treatment group.

To explore changes in presynaptic short-term plasticity, the baseline stimulation strength was set to 50% of the range between the minimum and maximum detectable fEPSP (as indicated by the appearance of a population spike during I/O curves) and a paired-pulse protocol was conducted where an initial stimulus was given, followed by a second pulse with an interpulse interval ranging from 25–300 ms, in sequentially increasing 25 ms intervals; each paired-pulse was separated by a 30-s interval. Paired-pulse ratio (PPR) was quantified as a percentage by dividing the slope of the second pulse by the slope of the first and expressed as an average for each interval by treatment group.

Voltage-Clamp Electrophysiology

To evaluate the effects of kindling on excitatory and inhibitory synaptic transmission onto DG granule cells (GCs), the whole cell patch clamp technique was used for voltage clamp recordings. An individual brain slice from dorsal hippocampus was transferred to a perfusion chamber (RC-27L, Warner Instruments, Hamden, CT, U.S.A.), held between 2 nylon nets, and perfused with oxygenated ACSF (2 mL/min) at room temperature. Patch electrodes (2.5–3.5 MΩ) were filled with the following internal solution (in mM): Cs Methanesulfonate (CH_3CsO_3S, 140.0), HEPES (10.0), EGTA (1.0), $CaCl_2$ (0.5), Glucose (10.0), ATP (2.0), GTP (0.5), and QX-314 (5.0)–300 mOsm, pH=7.30 (CsOH). Cells were "blindly" patched by forming tight seals (4–8 GΩ) with cells located in the GC layer of the DG. All recordings were performed using a Multiclamp 700 B amplifier and the pClamp 10 (Clampex) software package interfaced to a Digidata 1440 A digitizer (Molecular Devices); recordings were digitized at 10 kHz and filtered at 1 kHz. For recording spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs), cells were held at -70 mV and 0 mV, respectively. Access and membrane resistance, temperature, and holding current were monitored in 30-s increments; changes in these parameters >20% rendered a cell excluded from analysis. The amplitude, interevent interval (IEI), length, and time constants for sEPSCs and sIPSCs were analyzed using Mini Analysis Program v6.0.1 (Synaptosoft, Decatur, GA, U.S.A.) with the amplitude threshold for events set to 10 pA. All measurements were obtained by averaging amplitude, IEI, length or time constant from a 30-s recording epoch for each cell after recording for 15 min at either -70 mV or 0 mV. Measurements from each neuron were then averaged by experimental group.

Statistical Analysis

All numerical values were expressed as the mean ± SEM, and all error bars on graphs represent SEM, except for the immunofluorescence data represented by box and whisker plots. Metric task, immunofluorescence, and patch clamp data were compared using a two-tailed Student's *t* test. LTP and PPR data were compared using a repeated measure two-way ANOVA, and I/O curves were compared using repeated measure two-way ANOVA or linear regression. All data sets were tested for normality using Kolmogorov-Smirnov test. When $p < 0.05$, the appropriate nonparametric statistical test was employed. All statistical analyses were preformed using GraphPad Prism V5.0c (GraphPad Software, San Diego, CA); *, **, or *** indicate a *p*-value of < 0.05, < 0.01, and < 0.001, respectively.

Results

Corneal Kindling in C57BL/6 Mice

To model the development of seizures in C57BL/6 mice, we corneal kindled 2 cohorts of 20 mice each (10 kindled and 10 nonstimulated controls) by stimulating twice daily until each mouse had achieved at least 5 consecutive stage 5 seizures (Racine, 1972; Rowley and White, 2010). Our kindling paradigm illustrates that C57BL/6 male mice were fully kindled by stimulation #13 on day 7 (Figure 4.1). We also stimulated the mice on day 30 to ensure they were still kindled 3 days prior to beginning electrophysiology experiments. Despite using a weaker stimulus strength than what has been reported in corneal kindled CF1 mice, C57BL/6 mice kindled almost twice as quickly compared to CF1 mice (Rowley and White, 2010).

Corneal Kindled Mice Exhibit DG-Dependent Spatial Memory Deficits

Three days following cessation of kindling stimuli, corneal kindled and control mice (n = 10 per group) were habituated and tested for spatial memory impairments in the metric task (Lee et al., 2005; Goodrich-Hunsaker et al., 2008) (Figure 4.2A). Corneal kindled mice spent significantly less time exploring the objects in the spatially-novel test configuration compared to nonstimulated control mice (Figure 4.2B; control: 39.7 ± 5.4 s, kindled: 21.6 ± 4.4 s, $p < 0.05$, unpaired Student's t test). Additionally, corneal kindled mice had a significantly lower average RID compared to nonkindled mice (Figure 4.2C; control RID: 0.40 ± 0.06, kindled RID: -0.02 ± 0.11, $p < 0.01$, unpaired Student's t test). We also quantified distance traveled and found no significant differences between corneal kindled and control mice ($p > 0.05$, unpaired Student's t test). These results indicate that corneal kindled mice exhibited impaired spatial pattern processing when performing a behavioral test thought to rely on proper DG function.

Corneal Kindled Mice Exhibit Astrogliosis in the Absence of Overt Hippocampal Cell Loss

To determine whether corneal kindled mice exhibit cell loss or astrogliosis in the DG and CA1, we performed immunofluorescence staining for both neuronal nuclear antigen (NeuN), a neuron specific protein (Kim et al., 2009), and glial fibrillary acidic protein (GFAP), a marker for astrocyte activation (Anderson et al., 2014), on sections from both corneal kindled and nonkindled control mice (n = 12 sections from 4 mice / group). Quantification of NeuN reactivity in the DG granule cell (DGC) layer revealed there was no significant difference between kindled and control animals (Figure 4.3A–C),

whereas GFAP immunoreactivity was significantly increased (Figure 4.3D, GFAP area, control: 8050 ± 485.7 μm^2; kindled: 12271 ± 515.8 μm^2, $p < 0.001$, unpaired Student's t test). Additional quantification of NeuN reactivity in the hilus revealed no difference between kindled and control animals (Figure 4.3E, F).

We also quantified NeuN and GFAP expression in hippocampal area CA1. Quantification of NeuN reactivity revealed there was no significant difference between kindled and control animals (Figure 4.4A–C), whereas GFAP immunoreactivity was significantly increased (Figure 4.4D, GFAP area, control: 8945 ± 497.7 μm^2; kindled: 11296 ± 387.8 μm^2, $p < 0.01$, Mann-Whitney U test). Together, these results suggest that corneal-kindled C57BL/6 mice exhibit increased astrocyte activation in the DG and CA1 in the absence of overt neuron loss, consistent with results found in corneal kindled CF1 mice (Loewen et al., 2016).

Long-Term Potentiation is Attenuated in the Dentate Gyrus of Corneal Kindled Mice

To evaluate long-term synaptic plasticity in the DG of corneal kindled mice, we examined TBS-induced LTP at the medial perforant path to DGC synapse in acute hippocampal brain slices from kindled and control mice. Kindled mice exhibited significantly reduced LTP compared to control mice 30–60 min following TBS (Figure 4.5A, B; Control: 156% ± 8.9, n = 11 slices from 2 animals; kindled: 119.4% ± 4.7, n = 13 slices from 2 animals; $F_{(1,22)} = 16.98$, $p < 0.001$, two-way repeated-measures ANOVA). These results suggest that corneal kindled mice have impaired synaptic plasticity at excitatory synaptic inputs onto DGCs.

Dentate Gyrus Granule Cells Are Hyperexcitable in Corneal Kindled Mice

To further explore potential alterations in synaptic transmission in this model of partial epilepsy, we examined basal synaptic transmission at the medial perforant path to DGC synapse in acute hippocampal brain slices prepared from kindled and control animals (n = 14 slices from 2 animals / group). fEPSPs obtained during acquisition of I/O relationship curves revealed an increased postsynaptic response at equivalent stimulation strengths in kindled mice (Figure 4.6A, B; $F_{(1, 475)} = 475$, $p < 0.05$, two-way repeated measures ANOVA). To further explore this relationship and rule out potential confounds associated with stimulation, we plotted fEPSP response as a function of presynaptic input determined by fiber volley amplitudes. DGCs from kindled mice had a greater postsynaptic response for an equivalent presynaptic input over a range of increasing stimulation strengths (Figure 4.6C; $F_{(1, 1050)} = 130.521$, $p < 0.001$, linear regression). Moreover, DGCs from kindled mice required a lower stimulation strength (Figure 4.6D; average stimulation strength at first population spike, control: 6.63 ± 0.70 V, kindled: 4.46 ± 0.05 V, $p < 0.05$, Student's t test) and a smaller postsynaptic depolarization (Figure 4.6E; average slope of fEPSP at first population spike, control: -0.25 ± 0.05 mV / ms, kindled: -0.09 ± 0.01 mV / ms, $p < 0.01$, unpaired Student's t test) to achieve a population spike. Additionally, quantification of PPRs showed no differences in short-term synaptic plasticity at the medial perforant path to DGC synapse (Figure 4.6F, G). These results demonstrate that while the presynaptic perforant path input to the DG is unaltered, DGCs from kindled animals exhibit a larger postsynaptic depolarization in response to equivalent presynaptic input and require less presynaptic input and less

postsynaptic excitation to achieve a population spike. Taken together, these results suggest that DGCs from kindled mice are hyperexcitable.

Dentate Gyrus Granule Cells Exhibited Increased Membrane Resistances and Amplitudes of sEPSCs

Lastly, we wanted to determine the underlying mechanisms of increased excitability in DGCs using voltage-clamp electrophysiology. To record sEPSCs in acute hippocampal brain slices and minimize the contributions of GABAergic IPSCs, we held the membrane potential of DGCs at -70 mV. DGCs from kindled mice (n = 12 cells from 5 animals) had significantly increased membrane resistances compared to nonkindled control animals (n = 14 cells from 5 animals) (Figure 4.7A, B, representative traces; Table 4.1, control: 312.8 ± 21.5 MΩ, kindled: 431.2 ± 39.4 MΩ, $p < 0.05$, unpaired Student's t test). Although there were no differences in IEI, the amplitudes of sEPSCs in kindled mice were significantly increased compared to control mice (Table 4.1; control: 15.7 ± 0.6 pA, kindled: 17.9 ± 0.7 pA, $p < 0.05$ unpaired Student's t test). We also observed no difference in the decay rate or half-width of sEPSCs (Table 4.1).

After recording sEPSCs from DGCs at -70mV, we held the cells at 0 mV to minimize the contributions of glutamatergic EPSCs and recorded sIPSCs. We found no difference in IEI, amplitude, decay rate, or half-width of sIPSCs (Table 4.1). Together, these findings illustrate that corneal kindled mice have enhanced excitatory synaptic transmission in DGCs along with increased membrane resistances, again suggesting that DGCs are hyperexcitable.

Discussion

This study demonstrates that corneal kindled mice exhibit spatial pattern processing deficits in the absence of overt hippocampal cell loss. We also observed several changes in synaptic transmission, including attenuated LTP at the medial perforant path to DGC synapse, as well as an increased postsynaptic response and reduced threshold to population spike in DGCs during perforant path-DGC I/O relationships. Moreover, DGCs in kindled mice had greater membrane resistances and received sEPSCs that were greater in amplitude compared to nonkindled controls. We did not observe any differences in the frequency of excitatory or inhibitory spontaneous events in kindled animals, or the amplitudes of inhibitory synaptic events, and PPRs of fEPSPs were also unchanged. Together, these results suggest that DGCs in corneal kindled mice are hyperexcitable and have altered synaptic plasticity. Moreover, these alterations, observed in the absence of overt hippocampal neuron loss, may contribute to the observed DG-associated spatial memory impairments. Thus, corneal kindled mice may represent a novel model for the discovery of new treatments to ameliorate memory deficits in the patient with epilepsy or to determine whether a new therapeutic exacerbates cognitive dysfunction seen in the absence of treatment.

The significance of these findings stem from the idea that developing treatments for memory impairments in epilepsy may be hampered in animal models that exhibit overt hippocampal damage**.** It may be a more manageable endeavor to first develop therapies in animal models that exhibit memory deficits as a result of more subtle hippocampal pathophysiology. Moreover, not all patients with epilepsy who experience memory deficits have overt cell loss (Aikia et al., 1995; Lah et al., 2014), and cell loss

with HS are not always associated with memory impairment (Castro et al., 2013; Schmidt et al., 2015). Additionally, a growing body of literature suggests cognitive dysfunction in epilepsy may result from pathophysiology independent of cell death or the seizures themselves (Bender et al., 2013; Holmes, 2016). In a follow-up study to the report by Loewen et al. (2016) showing that corneal kindled CF1 mice exhibit no observable neuron loss in the hippocampus, we found that corneal kindled C57BL/6 mice exhibit DG-dependent spatial processing deficits and altered synaptic transmission and plasticity in the absence of overt hippocampal cell loss. Our results parallel findings in other kindling models also reporting neuronal hyperexcitability, impaired long-term synaptic plasticity, and hippocampal-dependent spatial memory deficits in the absence of overt hippocampal damage (Hannesson and Corcoran, 2000; Hannesson et al., 2001; Leung and Shen, 2006); however, these models are constrained by laborious implantation surgeries that cause a proinflammatory response in the brain. A noninvasive model of kindling that employs the chemoconvulsant pentylenetetrazol (PTZ) also produces hippocampal-dependent spatial memory impairment, increased neuronal excitability, and altered synaptic plasticity, but does produce hippocampal neuron loss (Pohle et al., 1997; Palizvan et al., 2001; Park et al., 2006; Mishra and Goel, 2012, 2013). Our results were found in a noninvasive cost-effective corneal kindling model of partial seizures that is not constrained by severe hippocampal damage and laborious implantation surgeries and is thus suitable to the development of new therapies for epilepsy-related memory deficits.

Considering the anatomy and physiology of proper DG function, DGC hyperexcitability in corneal kindled mice could have contributed to impaired performance during the metric task, which requires animals to discriminate spatial pattern differences

between 2 objects. The DG is the anatomical entranceway into the hippocampus that is critical for pattern separation, defined as the ability to discriminate similar synaptic inputs and transform them into dissimilar outputs (Amaral et al., 2007; Schmidt et al., 2012). DGCs accomplish this function, in part, by maintaining a hyperpolarized resting membrane potential and low firing probability – and thus, gate information flow into the hippocampus (Coulter and Carlson, 2007; Hsu, 2007). Moreover, pathological increases in DG excitability have been associated with impaired performance during pattern separation tasks in rodents (Jinde et al., 2012; Park et al., 2015) and humans alike (Bakker et al., 2012); remarkably, attenuating aberrantly increased DG excitability restored pattern separation ability in these studies. These findings support the idea that DGC hyperexcitability in corneal kindled mice could impair spatial pattern processing during the metric task and may represent a valid therapeutic target for the treatment of memory impairment in epilepsy.

The underlying causes of neuronal hyperexcitability in models of epilepsy are numerous and frequently investigated. Studies that employ direct electrical stimulation or chemoconvulsants to produce status epilepticus and subsequent spontaneous seizures in rodents report increased excitability in the hippocampus that is thought to facilitate seizure activity and contribute to memory dysfunction, but is often attributed to gross anatomical changes such as neuronal loss – primarily the loss of inhibitory interneurons that synapse onto DGCs, or the loss of excitatory mossy cells within the hilus of the DG that drive inhibition, as well as aberrant neuronal sprouting and reorganization (Cossart et al., 2001; Hannesson et al., 2001; Sloviter et al., 2003; Harvey and Sloviter, 2005; Leung and Shen, 2006; Sloviter et al., 2006; Sun et al., 2007; Chauviere et al., 2009; Jinde et al.,

2012). In contrast, electrical kindling models also exhibit hippocampal-dependent memory impairment and neuronal hyperexcitability, but usually in the absence of overt hippocampal cell loss (Hannesson and Corcoran, 2000), which suggests more subtle changes in hippocampal physiology underlie these deficits. Although the underlying causes of DG-mediated memory impairment in the present study are likely multifactorial, we observed DGC hyperexcitability in the absence of overt hippocampal cell loss, alterations to PPR of fEPSPs at the perforant path-DGC synapse, and the frequency of spontaneous excitatory or inhibitory synaptic transmission – which suggests that DGC hyperexcitability is likely due to changes within DGCs as opposed to the gain or loss of presynaptic drive. Besides increased membrane resistances, the increased expression, insertion, and/or phosphorylation of glutamate receptors on DGCs may have contributed to the increased postsynaptic response observed in DGCs, as similar changes in glutamate receptor activity and function has been reported in other epilepsy models (Mathern et al., 1997; Lopes et al., 2013). We also cannot rule out the possibility that increased activation of astrocytes in the DG, observed in the form of increased GFAP immunoreactivity, may have also contributed to DGC hyperexcitability, since activation of astrocytes has been shown to be sufficient to alter glutamate uptake by astrocytes and cause seizures in mice (Takahashi et al., 2010; Robel et al., 2015). Future experiments are needed to better understand the causes of DGC hyperexcitability in corneal kindled mice.

Besides DGC hyperexcitability, many other alterations may have contributed to DG-associated memory dysfunction observed in corneal kindled mice. For example, LTP in the DG has been shown to be critical for the performance of pattern separation tasks in mice (McHugh et al., 2007). Thus, our observation of impaired LTP at the perforant path-

DGC synapse, which parallels findings in tissue from humans with epilepsy (Beck et al., 2000), may have also contributed to DG-mediated memory impairments in corneal kindled mice. Although beyond the scope of this study, myriad pathological changes may have contributed to attenuated LTP in the DG of corneal kindled mice. For instance, the development of seizures in response to initially subconvulsive stimulations during kindling occurs via synaptic and cellular mechanisms similar to those of LTP (Albensi et al., 2007)`. Therefore, corneal kindling may have saturated the capability for further synaptic potentiation at the perforant-DGC synapse via metaplastic changes, as suggested by others (Huang et al., 1992; McIntyre et al., 2002; Meador, 2007). However, limited conclusions regarding the cause and effect relationships of attenuated LTP and DGC hyperexcitability can be drawn from the present study. We also cannot rule out the possibility that changes in adult hippocampal neurogenesis may have contributed to DG-mediated memory deficits observed in corneal kindled mice as attenuating aberrantly increased hippocampal neurogenesis has been shown to prevent cognitive impairment in animal models of epilepsy (Jessberger et al., 2007a; Jessberger et al., 2007b; Fournier et al., 2013; Botterill et al., 2015). Future experiments are needed to further explore the relationships between DGC hyperexcitability, synaptic plasticity, and hippocampal neurogenesis in cognitive deficits in corneal kindled mice.

Regardless of the underlying cause for DG-mediated memory impairment in corneal kindled mice, a growing body of work suggests that the DG supports performance during pattern separation tasks by “fine-tuning” the balance of excitatory and inhibitory networks and disrupting this balance impairs DG-mediated task performance (Piatti et al., 2013; Park et al., 2015). Although excitatory-inhibitory

imbalance is a key feature in models of epilepsy, and studying the underlying causes in models of epilepsy can provide insight into the pathological mechanisms that give rise to seizures and associated cognitive comorbidities, it may be more manageable to study memory impairment in seizure models without severe hippocampal damage, where more subtle changes in hippocampal physiology underlie memory deficits. Towards this end, corneal kindled mice represent a noninvasive, cost-effective rapid screening model of local seizures that secondarily generalize. Targeting DGC hyperexcitability and impaired synaptic plasticity may be useful in the discovery of novel treatments for epilepsy-related memory deficits.

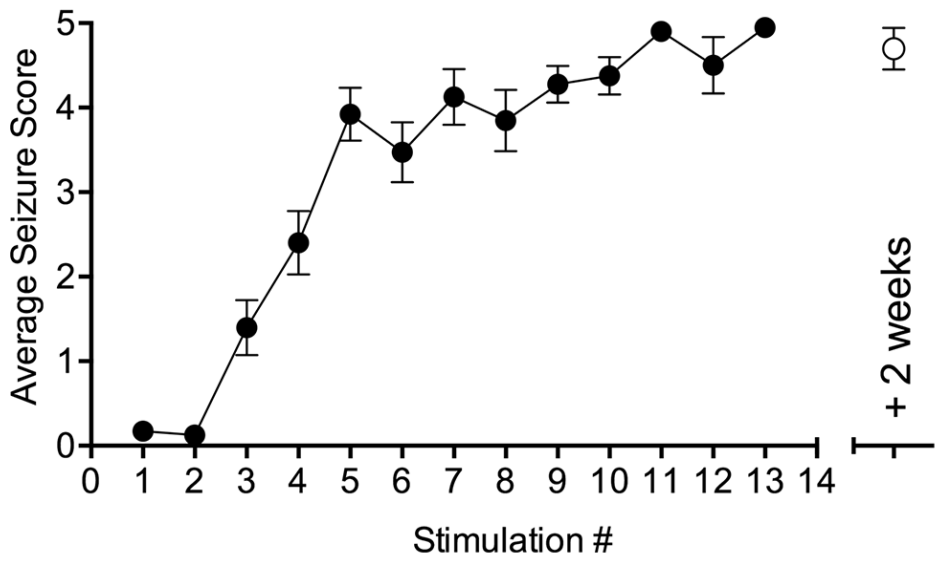


Figure 4.1. Corneal kindling curve in mice. Male C57BL/6 mice (n = 20, 15–20 g) were stimulated twice daily and their average seizures scores determined according to the Racine scale. Mice took approximately 13 stimulations (7 days) to become fully kindled. Mice were still fully kindled after 2 weeks without stimulation.

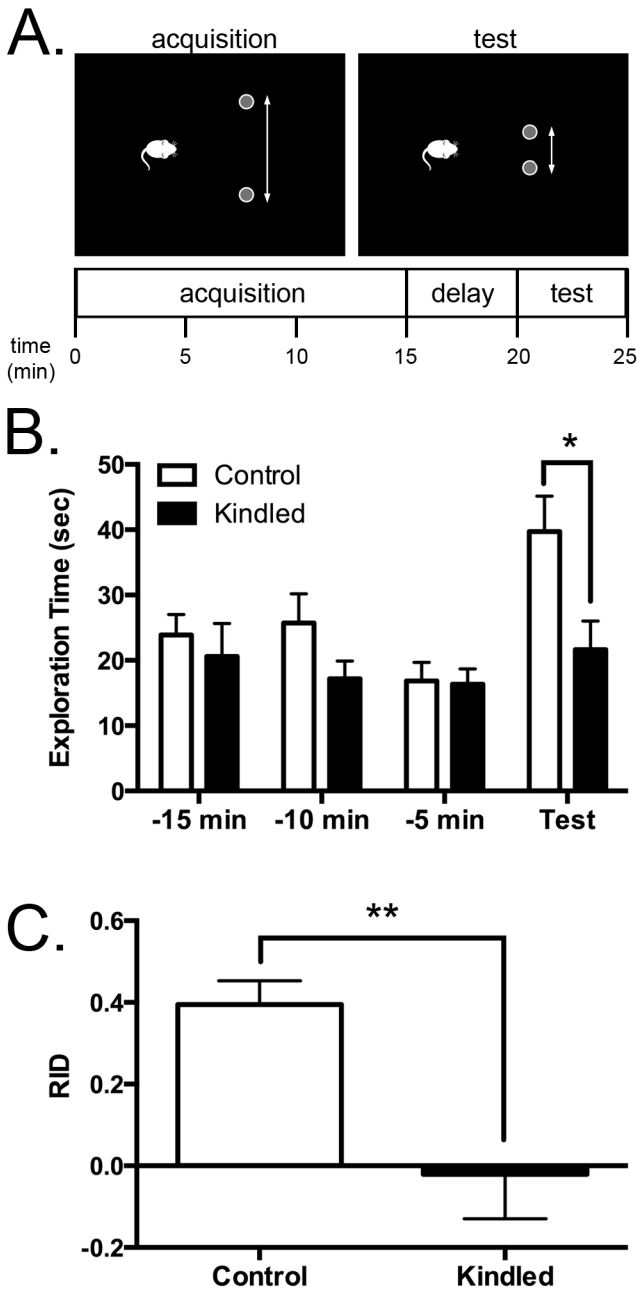


Figure 4.2. Corneal kindled mice exhibit DG-dependent memory impairment during the metric task. A) Schematic illustrating the metric task. B) Total object exploration time averaged by phase. There were no significant differences between the control and kindled mice until the test phase, when kindled mice spent significantly less time exploring the objects compared to control mice ($p < 0.05$, Student's t test, $n = 10$ per treatment group). C) Average recognition index (RID) in kindled mice was significantly less than the average RID in control mice (** $p < 0.01$, Student's t test).

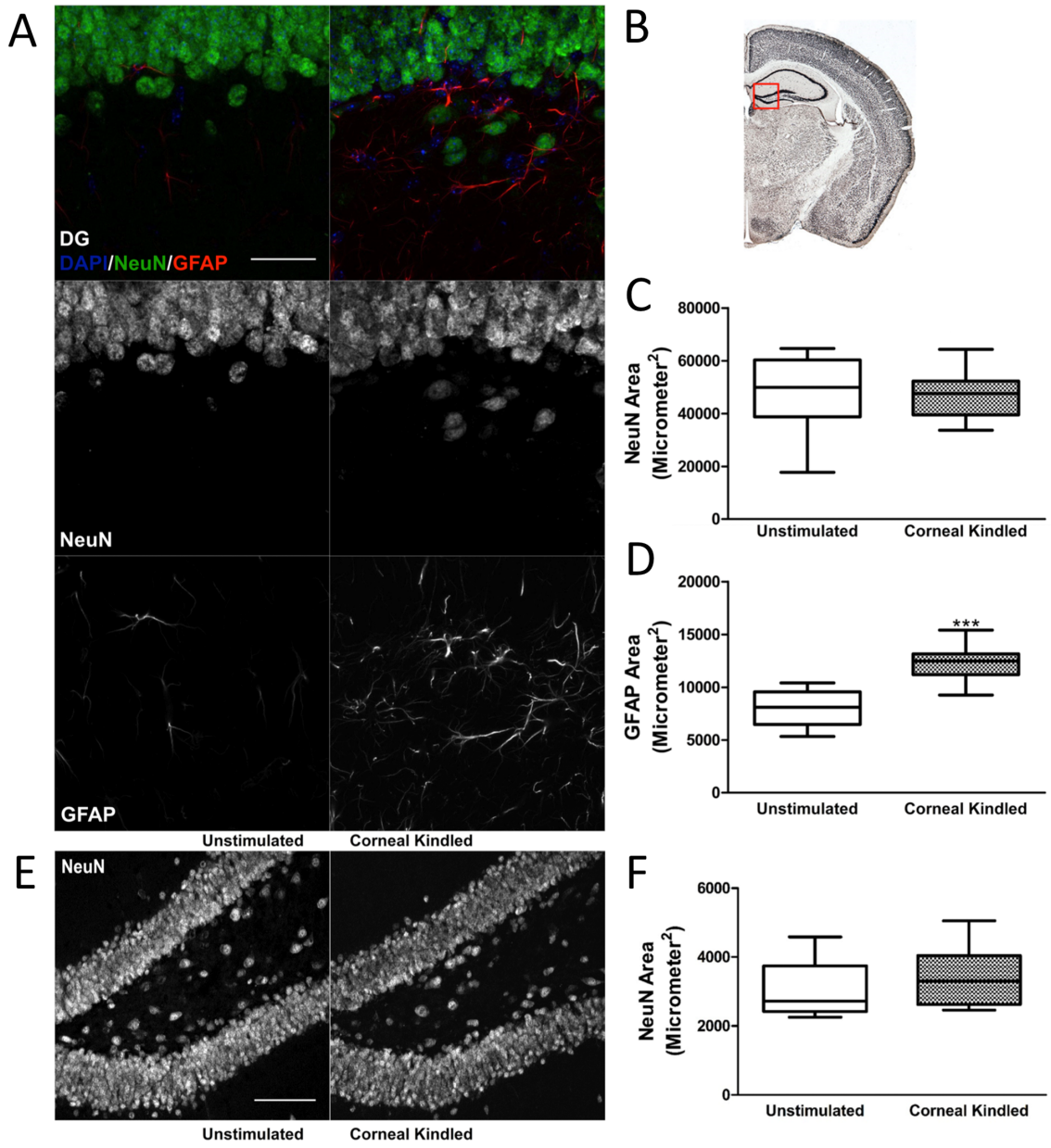


Figure 4.3. Corneal kindled mice exhibit increased activation of astrocytes in the DG in the absence of overt neuronal cell loss. A) Representative images of NeuN and GFAP staining from the DG. B) The region of interest that was quantified. Scale bar: 50 µm. C) Quantification of NeuN staining in the DG revealed no significant differences (n = 12 sections from 4 mice per group), however, D) quantification of GFAP staining revealed increased expression in kindled mice ($p < 0.001$, Student's t test). E) Representative images of NeuN staining used to quantify staining in the hilar region of the DG. Scale bar: 100 µm. F) Quantification of NeuN staining in the hilus revealed no significant differences between kindled and control animals.

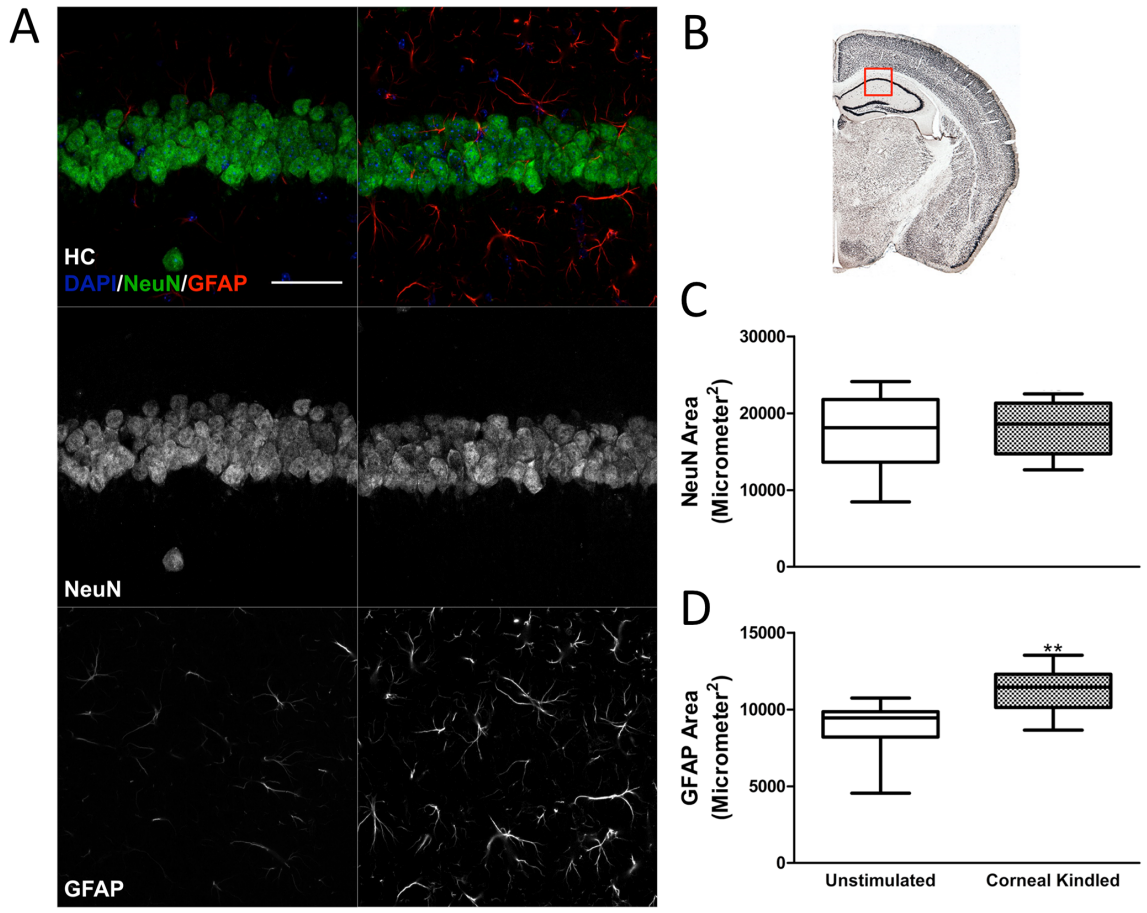


Figure 4.4. Corneal kindled mice exhibit increased astrocyte activation in hippocampal area CA1 in the absence of overt neuronal cell loss. A) Representative images of NeuN and GFAP staining in CA1 and, B) the region of interest that was quantified. Scale bar: 50 µm. C) Quantification of NeuN staining in CA1 revealed no significant differences (n = 12 sections from 4 mice per group), however, D) quantification of GFAP staining revealed increased expression in kindled mice (** $p < 0.01$, Mann-Whitney U test).

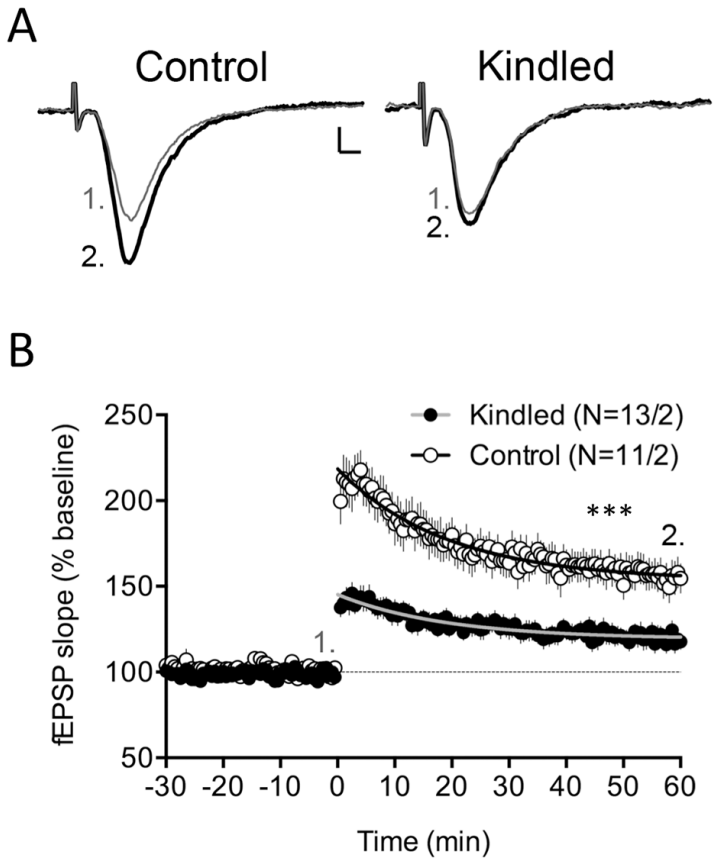
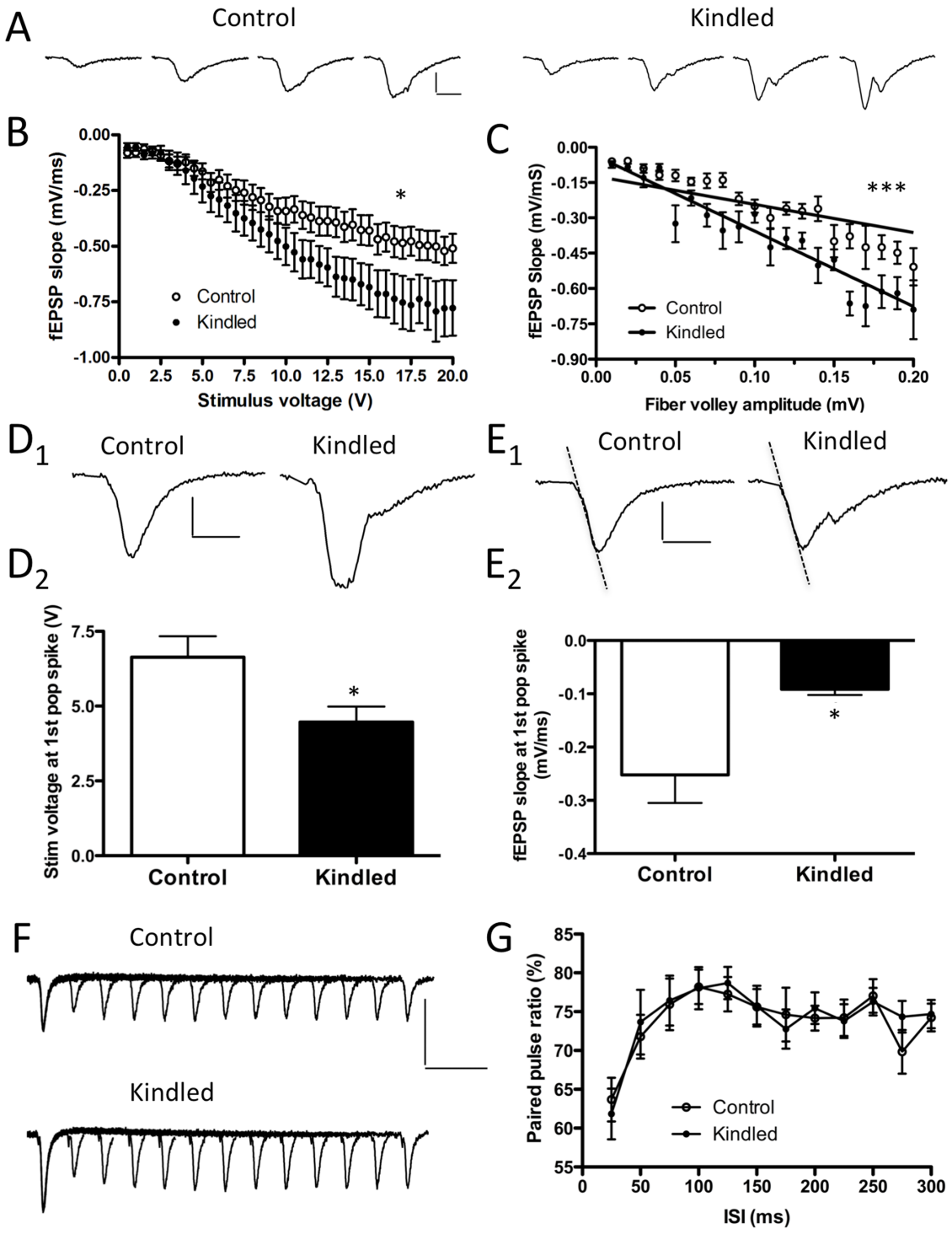


Figure 4.5. Brain slices from corneal kindled mice exhibit attenuated LTP along the perforant path. A) Representative fEPSPs from 30 s prior to (1, gray) and 60 min post-TBS (2, black) in control and kindled mice. Scale bars 0.25 mV, 2 ms. are applicable to both control and kindled traces. B) fEPSP slope as a function of time from control (open circles, n = 11 slices from 2 animals) and kindled animals (closed circles, n = 13 from 2 animals) conducted in the presence of 10 μM PTX. fEPSP amplitudes are normalized to the average of the last 4 fEPSPs before TBS. TBS occurred at time = 0 min. Kindled mice exhibited significantly attenuated LTP between 30 and 60 min post-TBS (*** $p <$ 0.001, two-way ANOVA).

Figure 4.6. Input / output curves along the perforant path indicate that DGCs are hyperexcitable. A) Representative fEPSPs from Input / output (I/O) relationships in both control and kindled animals. From left to right, each fEPSP is a response from a 5, 10, 15, or 20 V stimulus. B) I/O curves by experimental group. fEPSP slopes are shown as a function of stimulus strength. Kindled mice exhibited an increased postsynaptic response compared to control animals (* $p < 0.05$, two-way repeated measures ANOVA, $n = 14$ slices from 2 animals per treatment group). C) I/O curves as a function of fiber volley amplitudes. Kindled mice exhibited an increased postsynaptic response compared to control animals (*** $p < 0.001$, linear regression; $n = 14$ slices from 2 animals, per group). D) DGCs of kindled mice develop a population spike in response to lower stimulus strengths. D_1) Representative images from a 5.5 V stimulus. Note the population spike is only present in the representative trace from the kindled animal. D_2) Average stimulus voltage when a population spike was first detected during I/O relationships was lower in kindled animals ($p < 0.05$, Student's t test). E) Action potentials in DGCs of kindled mice are triggered by comparatively smaller postsynaptic depolarizations as indicated by the slope of the fEPSP when the first population spike was identified. E_1) Representative fEPSPs. The dashed lines illustrate two fEPSPs with the same slope. Note that the population spike is only present in the representative trace from the kindled animal. E_2) Average slope of the fEPSP when a population spike was first detected during I/O relationships was lower in kindled animals ($p < 0.05$, Student's t test). F) Representative traces of fEPSPs evoked by paired-pulse stimulations of varying interstimulus intervals, and *G*) paired-pulse ratios (PPRs) plotted as a function of the (ISI). PPRs did not differ between kindled and control animals. Scale bars for fEPSPs: 0.5 mV, 5 ms; PPR: 0.5 mV, 50 ms.


A
Control
Kindled
B
fEPSP slope (mV/ms)
0.00
-0.25
-0.50
-0.75
-1.00
0.0 2.5 5.0 7.5 10.0 12.5 15.0 17.5 20.0
Stimulus voltage (V)
Control
Kindled
*
C
fEPSP Slope (mV/mS)
0.00
-0.15
-0.30
-0.45
-0.60
-0.75
-0.90
0.00 0.05 0.10 0.15 0.20
Fiber volley amplitude (mV)
Control
Kindled
***
D1
Control
Kindled
D2
Stim voltage at 1st pop spike (V)
7.5
5.0
2.5
0.0
Control
Kindled
*
E1
Control
Kindled
E2
fEPSP slope at 1st pop spike (mV/ms)
0.0
-0.1
-0.2
-0.3
-0.4
Control
Kindled
*
F
Control
Kindled
G
Paired pulse ratio (%)
85
80
75
70
65
60
55
0 50 100 150 200 250 300
ISI (ms)
Control
Kindled

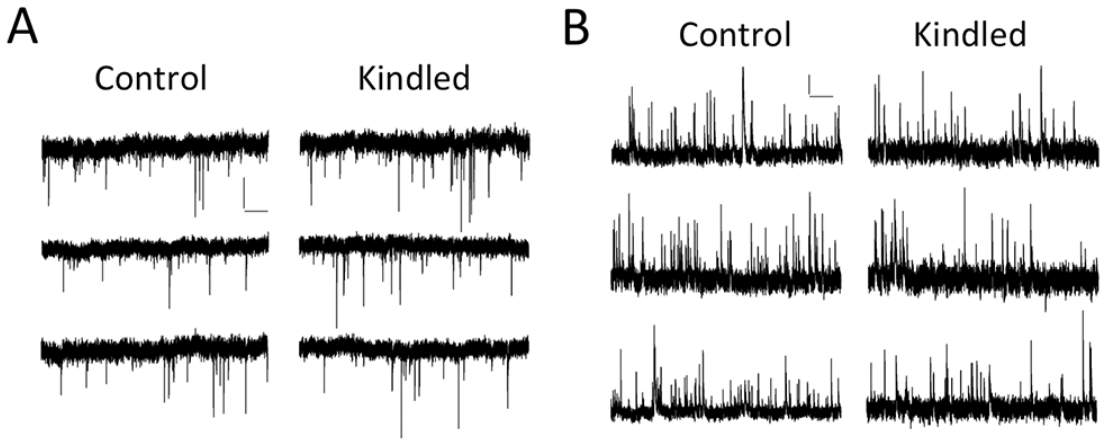


Figure 4.7. DGCs from kindled mice receive sEPSCs with increased amplitudes but did not exhibit any changes in spontaneous inhibitory synaptic transmission. A) Representative sEPSCs, and B) sIPSCs from control and kindled mice when DGCs are held at -70 mV or 0 mV, respectively. Scale bars: 10 pA, 1 s.

Table 4.1

Results from voltage-clamp experiments showing the effects of corneal kindling on excitatory and inhibitory postsynaptic currents in dentate granule cells.

Measurements are shown as mean ± SEM. R_m = membrane resistance; IEI = interevent interval; Amp = amplitude (* $p < 0.05$, unpaired Student's *t* test, $n = 12$ cells from 5 kindled animals, and $n = 14$ cells from 5 control animals).

		R_m (MΩ)	IEI (ms)	Amp (pA)	Decay (ms)	Half-width (ms)
Excitatory (-70mV)	Control	312.8 ± 21.5	902.5 ± 151.0	15.7 ± 0.6	6.5 ± 0.7	6.4 ± 0.7
	Kindled	431.2 ± 39.4*	1119.0 ± 166.3	17.9 ± 0.7*	6.4 ± 0.7	6.0 ± 0.6
Inhibitory (0 mV)	Control	127.0 ± 20.2	201.1 ± 23.5	22.0 ± 1.4	11.8 ± 0.5	10.1 ± 0.5
	Kindled	153.9 ± 19.8	191.8 ± 28.0	22.7 ± 0.9	11.7 ± 0.7	10.4 ± 0.7

References

Aikia M, Kalviainen R, Riekkinen PJ (1995) Verbal learning and memory in newly diagnosed partial epilepsy. Epilepsy Res 22:157-164.

Aikia M, Salmenpera T, Partanen K, Kalviainen R (2001) Verbal memory in newly diagnosed patients and patients with chronic left temporal lobe epilepsy. Epilepsy Behav 2:20-27.

Albensi BC, Oliver DR, Toupin J, Odero G (2007) Electrical stimulation protocols for hippocampal synaptic plasticity and neuronal hyper-excitability: are they effective or relevant? Exp Neurol 204:1-13.

Amaral DG, Scharfman HE, Lavenex P (2007) The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Prog Brain Res 163:3-22.

Anderson MA, Ao Y, Sofroniew MV (2014) Heterogeneity of reactive astrocytes. Neurosci Lett 565:23-29.

Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, Yassa MA, Bassett SS, Shelton AL, Gallagher M (2012) Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron 74:467-474.

Beck H, Goussakov IV, Lie A, Helmstaedter C, Elger CE (2000) Synaptic plasticity in the human dentate gyrus. J Neurosci 20:7080-7086.

Bender AC, Natola H, Ndong C, Holmes GL, Scott RC, Lenck-Santini PP (2013) Focal Scn1a knockdown induces cognitive impairment without seizures. Neurobiol Dis 54:297-307.

Botterill JJ, Brymer KJ, Caruncho HJ, Kalynchuk LE (2015) Aberrant hippocampal neurogenesis after limbic kindling: Relationship to BDNF and hippocampal-dependent memory. Epilepsy Behav 47:83-92.

Brandt C, Ebert U, Loscher W (2004) Epilepsy induced by extended amygdala-kindling in rats: lack of clear association between development of spontaneous seizures and neuronal damage. Epilepsy Res 62:135-156.

Castro LH, Silva LC, Adda CC, Banaskiwitz NH, Xavier AB, Jorge CL, Valerio RM, Nitrini R (2013) Low prevalence but high specificity of material-specific memory impairment in epilepsy associated with hippocampal sclerosis. Epilepsia 54:1735-1742.

Chauviere L, Rafrafi N, Thinus-Blanc C, Bartolomei F, Esclapez M, Bernard C (2009) Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. J Neurosci 29:5402-5410.

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben-Ari Y, Esclapez

M, Bernard C (2001) Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nat Neurosci 4:52-62.

Coulter DA, Carlson GC (2007) Functional regulation of the dentate gyrus by GABA-mediated inhibition. Prog Brain Res 163:235-243.

Covolan L, Mello LE (2000) Temporal profile of neuronal injury following pilocarpine or kainic acid-induced status epilepticus. Epilepsy Res 39:133-152.

de Lanerolle NC, Lee TS, Spencer DD. (2012). Histopathology of Human Epilepsy. In J. L. Noebels, M. Avoli, M. A. Rogawski, R. W. Olsen & A. V. Delgado-Escueta (Eds.), *Jasper's Basic Mechanisms of the Epilepsies* (4th ed.). Bethesda (MD).

Ennaceur A (2010) One-trial object recognition in rats and mice: methodological and theoretical issues. Behav Brain Res 215:244-254.

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav Brain Res 31:47-59.

Fournier NM, Botterill JJ, Marks WN, Guskjolen AJ, Kalynchuk LE (2013) Impaired recruitment of seizure-generated neurons into functional memory networks of the adult dentate gyrus following long-term amygdala kindling. Exp Neurol 244:96-104.

Friedman LK, Pellegrini-Giampietro DE, Sperber EF, Bennett MV, Moshe SL, Zukin RS (1994) Kainate-induced status epilepticus alters glutamate and GABAA receptor gene expression in adult rat hippocampus: an in situ hybridization study. J Neurosci 14:2697-2707.

Giovagnoli AR, Avanzini G (2000) Quality of life and memory performance in patients with temporal lobe epilepsy. Acta Neurol Scand 101:295-300.

Goodrich-Hunsaker NJ, Hunsaker MR, Kesner RP (2008) The interactions and dissociations of the dorsal hippocampus subregions: how the dentate gyrus, CA3, and CA1 process spatial information. Behav Neurosci 122:16-26.

Groticke I, Hoffmann K, Loscher W (2008) Behavioral alterations in a mouse model of temporal lobe epilepsy induced by intrahippocampal injection of kainate. Exp Neurol 213:71-83.

Hannesson DK, Corcoran ME (2000) The mnemonic effects of kindling. Neurosci Biobehav Rev 24:725-751.

Hannesson DK, Mohapel P, Corcoran ME (2001) Dorsal hippocampal kindling selectively impairs spatial learning/short-term memory. Hippocampus 11:275-286.

Harvey BD, Sloviter RS (2005) Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. J Comp Neurol 488:442-463.

Hermann BP, Seidenberg M, Dow C, Jones J, Rutecki P, Bhattacharya A, Bell B (2006) Cognitive prognosis in chronic temporal lobe epilepsy. Ann Neurol 60:80-87.

Hesdorffer DC, Logroscino G, Benn EK, Katri N, Cascino G, Hauser WA (2011) Estimating risk for developing epilepsy: a population-based study in Rochester, Minnesota. Neurology 76:23-27.

Holmes GL (2016) Effect of seizures on the developing brain and cognition. Semin Pediatr Neurol 23:120-126.

Hrabok M, Sherman EM, Bello-Espinosa L, Hader W (2013) Memory and health-related quality of life in severe pediatric epilepsy. Pediatrics 131:e525-532.

Hsu D (2007) The dentate gyrus as a filter or gate: a look back and a look ahead. Prog Brain Res 163:601-613.

Huang YY, Colino A, Selig DK, Malenka RC (1992) The influence of prior synaptic activity on the induction of long-term potentiation. Science 255:730-733.

Jessberger S, Nakashima K, Clemenson GD, Jr., Mejia E, Mathews E, Ure K, Ogawa S, Sinton CM, Gage FH, Hsieh J (2007a) Epigenetic modulation of seizure-induced neurogenesis and cognitive decline. J Neurosci 27:5967-5975.

Jessberger S, Zhao C, Toni N, Clemenson GD, Jr., Li Y, Gage FH (2007b) Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. J Neurosci 27:9400-9407.

Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K (2012) Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron 76:1189-1200.

Kim KK, Adelstein RS, Kawamoto S (2009) Identification of neuronal nuclei (NeuN) as Fox-3, a new member of the Fox-1 gene family of splicing factors. J Biol Chem 284:31052-31061.

Lah S, Mohamed A, Thayer Z, Miller L, Diamond K (2014) Accelerated long-term forgetting of verbal information in unilateral temporal lobe epilepsy: Is it related to structural hippocampal abnormalities and/or incomplete learning? J Clin Exp Neuropsychol 36:158-169.

Lee I, Hunsaker MR, Kesner RP (2005) The role of hippocampal subregions in detecting spatial novelty. Behav Neurosci 119:145-153.

Leung LS, Shen B (2006) Hippocampal CA1 kindling but not long-term potentiation disrupts spatial memory performance. Learn Mem 13:18-26.

Loewen JL, Barker-Haliski ML, Dahle EJ, White HS, Wilcox KS (2016) Neuronal injury, gliosis, and glial proliferation in two models of temporal lobe epilepsy. J

Neuropathol Exp Neurol 75:366-378.

Lopes MW, Soares FM, de Mello N, Nunes JC, Cajado AG, de Brito D, de Cordova FM, da Cunha RM, Walz R, Leal RB (2013) Time-dependent modulation of AMPA receptor phosphorylation and mRNA expression of NMDA receptors and glial glutamate transporters in the rat hippocampus and cerebral cortex in a pilocarpine model of epilepsy. Exp Brain Res 226:153-163.

Matagne A, Klitgaard H (1998) Validation of corneally kindled mice: a sensitive screening model for partial epilepsy in man. Epilepsy Res 31:59-71.

Mathern GW, Bertram EH, 3rd, Babb TL, Pretorius JK, Kuhlman PA, Spradlin S, Mendoza D (1997) In contrast to kindled seizures, the frequency of spontaneous epilepsy in the limbic status model correlates with greater aberrant fascia dentata excitatory and inhibitory axon sprouting, and increased staining for N-methyl-D-aspartate, AMPA and GABA(A) receptors. Neuroscience 77:1003-1019.

McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK, Lowell BB, Fanselow MS, Wilson MA, Tonegawa S (2007) Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampal network. Science 317:94-99.

McIntyre DC, Poulter MO, Gilby K (2002) Kindling: some old and some new. Epilepsy Res 50:79-92.

Meador KJ (2007) The basic science of memory as it applies to epilepsy. Epilepsia 48 Suppl 9:23-25.

Mishra A, Goel RK (2012) Age dependent learning and memory deficit in Pentylenetetrazol kindled mice. Eur J Pharmacol 674:315-320.

Mishra A, Goel RK (2013) Psychoneurochemical investigations to reveal neurobiology of memory deficit in epilepsy. Neurochem Res 38:2503-2515.

Palizvan MR, Fathollahi Y, Semnanian S, Hajezadeh S, Mirnajafizadh J (2001) Differential effects of pentylenetetrazol-kindling on long-term potentiation of population excitatory postsynaptic potentials and population spikes in the CA1 region of rat hippocampus. Brain Res 898:82-90.

Park EH, Burghardt NS, Dvorak D, Hen R, Fenton AA (2015) Experience-dependent regulation of dentate gyrus excitability by adult-born granule cells. J Neurosci 35:11656-11666.

Park JH, Cho H, Kim H, Kim K (2006) Repeated brief epileptic seizures by pentylenetetrazole cause neurodegeneration and promote neurogenesis in discrete brain regions of freely moving adult rats. Neuroscience 140:673-684.

Piatti VC, Ewell LA, Leutgeb JK (2013) Neurogenesis in the dentate gyrus: carrying the message or dictating the tone. Front Neurosci 7:50.

Pohle W, Becker A, Grecksch G, Juhre A, Willenberg A (1997) Piracetam prevents pentylenetetrazol kindling-induced neuronal loss and learning deficits. Seizure 6:467-474.

Potschka H, Loscher W (1999) Corneal kindling in mice: behavioral and pharmacological differences to conventional kindling. Epilepsy Res 37:109-120.

Racine RJ (1972) Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 32:281-294.

Rao MS, Hattiangady B, Reddy DS, Shetty AK (2006) Hippocampal neurodegeneration, spontaneous seizures, and mossy fiber sprouting in the F344 rat model of temporal lobe epilepsy. J Neurosci Res 83:1088-1105.

Robel S, Buckingham SC, Boni JL, Campbell SL, Danbolt NC, Riedemann T, Sutor B, Sontheimer H (2015) Reactive astrogliosis causes the development of spontaneous seizures. J Neurosci 35:3330-3345.

Rowley NM, White HS (2010) Comparative anticonvulsant efficacy in the corneal kindled mouse model of partial epilepsy: Correlation with other seizure and epilepsy models. Epilepsy Res 92:163-169.

Schmidt B, Marrone DF, Markus EJ (2012) Disambiguating the similar: the dentate gyrus and pattern separation. Behav Brain Res 226:56-65.

Schmidt CS, Lassonde M, Gagnon L, Sauerwein CH, Carmant L, Major P, Paquette N, Lepore F, Gallagher A (2015) Neuropsychological functioning in children with temporal lobe epilepsy and hippocampal atrophy without mesial temporal sclerosis: a distinct clinical entity? Epilepsy Behav 44:17-22.

Sloviter RS, Zappone CA, Harvey BD, Bumanglag AV, Bender RA, Frotscher M (2003) "Dormant basket cell" hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. J Comp Neurol 459:44-76.

Sloviter RS, Zappone CA, Harvey BD, Frotscher M (2006) Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyper-inhibition in chronically epileptic rats. J Comp Neurol 494:944-960.

Stopps M, Allen N, Barrett R, Choudhury HI, Jarolimek W, Johnson M, Kuenzi FM, Maubach KA, Nagano N, Seabrook GR (2004) Design and application of a novel brain slice system that permits independent electrophysiological recordings from multiple slices. J Neurosci Methods 132:137-148.

Sun C, Mtchedlishvili Z, Bertram EH, Erisir A, Kapur J (2007) Selective loss of dentate hilar interneurons contributes to reduced synaptic inhibition of granule cells in an electrical stimulation-based animal model of temporal lobe epilepsy. J Comp Neurol

500:876-893.

Takahashi DK, Vargas JR, Wilcox KS (2010) Increased coupling and altered glutamate transport currents in astrocytes following kainic-acid-induced status epilepticus. Neurobiol Dis 40:573-585.

Tooyama I, Bellier JP, Park M, Minnasch P, Uemura S, Hisano T, Iwami M, Aimi Y, Yasuhara O, Kimura H (2002) Morphologic study of neuronal death, glial activation, and progenitor cell division in the hippocampus of rat models of epilepsy. Epilepsia 43 Suppl 9:39-43.

West PJ, Saunders GW, Remigio GJ, Wilcox KS, White HS (2014) Antiseizure drugs differentially modulate theta-burst induced long-term potentiation in C57BL/6 mice. Epilepsia 55:214-223.

CHAPTER 5

DISCUSSION

Conclusions

The work in this dissertation investigated the neurophysiology and behavioral pharmacology of memory enhancement and memory deficits. Chapter 2 examined 5-HT_6 receptor localization and physiology in the dentate gyrus (DG) to better understand how 5-HT_6 receptor antagonists produce their widely reported nootropic effects, and Chapter 3 tested whether 5-HT_6 receptor antagonists exhibit procognitive and anticonvulsant properties in a transgenic model of Alzheimer's disease (AD). Lastly, Chapter 4 found spatial pattern processing deficits and associated hyperexcitability that may contribute to cognitive dysfunction in corneal kindled mice. For a broader perspective on these results, I will evaluate the global implications of the results obtained in these studies, which suggest that targeting disinhibition and hyperexcitability may represent worthwhile approaches for the treatment of memory deficits in epilepsy and AD. Both of these phenomena and their roles in memory enhancement and memory impairment have been studied extensively. Therefore, this discussion will begin by evaluating the top-down and bottom-up approaches that guided the research in this dissertation and highlight the pros and cons of targeting disinhibition and hyperexcitability for the treatment of memory deficits in disease. I will then discuss how targeting disinhibition and hyperexcitability

for the treatment of memory deficits in disease are inextricably linked, highlighting the idea that treatments for cognitive dysfunction in disease should be *both* procognitive and anticonvulsant. Lastly, I will discuss how these themes instruct future therapy development and fit into the armamentarium of treatments that may be needed to combat cognitive dysfunction in disease.

The Pros and Cons of a Top-Down Approach to New Therapy Development

A Roadmap to Novel Therapeutic Targets

As it relates to the work in this dissertation, the top-down approach sought to provide an anatomical and physiological roadmap for novel treatments based on existing treatments already shown to be effective at ameliorating disease symptoms. The rationale is that a better understanding of existing treatments will allow us to "fine-tune" their effects, and potentially highlight new therapeutic targets. Attempts to reverse-engineer existing treatments holds promise as a research approach that is perhaps best exemplified by the historical study of antipsychotics and antidepressants.

Following the cloning of monoaminergic receptors, attempts to reverse-engineer antipsychotics and tricyclic antidepressants using high throughput receptor screening assays revealed that antipsychotic and tricyclic antidepressant drugs had high affinity for a variety of monoaminergic receptors. Chemical libraries were soon screened for ligands selective for individual receptor subtypes. One target that emerged from these efforts is the serotonin 5-HT_6 receptor subtype (Roth et al., 1994). In the following years, 5-HT_6 receptor selective ligands were developed and tested in behavioral assays revealing

remarkable results – particularly the antagonists, which have been shown to improve cognition on a number of hippocampal, striatal, and prefrontal cortex-dependent tasks (Fone, 2008). Moreover, 5-HT_6 receptor antagonists also exhibit antidepressant and anxiolytic properties, and are now implicated for their potential use in the treatment of schizophrenia, anxiety disorders, seizures, and the latest – obesity (Hirst et al., 2006; Heal et al., 2008; Schechter et al., 2008; Yun and Rhim, 2011). On the strength of the preclinical data, 5-HT_6 receptor antagonists advanced to clinical trials in patients with AD, showing modest but beneficial effects on cognitive function with relatively few adverse events (Maher-Edwards et al., 2010; Maher-Edwards et al., 2011; Wilkinson et al., 2014). As a result of their widely reported nootropic effects, researchers are now trying to reverse-engineer 5-HT_6 receptor physiology and function.

Results in this dissertation, and a previously published body of literature, suggest that 5-HT_6 receptor antagonists exert their procognitive effects by attenuating inhibition and facilitating information flow through excitatory networks – a neural phenomenon known as disinhibition (Dawson et al., 2001; Woolley et al., 2004; Fone, 2008; Schechter et al., 2008; Codony et al., 2011; Ramirez, 2013). Moreover, results obtained in Chapter 2 are, to my knowledge, the first to suggest that 5-HT_6 receptors bidirectionally modulate inhibition in the DG, and may do so via their direct effects on mossy cells. Thus, attempts to reverse-engineer antipsychotics and antidepressants led researchers to the remarkable effects of 5-HT_6 receptor ligands on cognition that are now being tested in clinical trials, and attempts to reverse-engineer 5-HT_6 receptor effects on cognition are now highlighting potential cell types that may be targeted for the treatment of cognitive dysfunction, thereby illustrating that the top-down approach may be useful for the

development of novel therapies.

The Top-Down Approach Relies on the Bottom-Up

Our understanding of brain function in health and disease has undoubtedly made great progress but is far from complete. One limitation to reverse-engineering 5-HT_6 receptor antagonists' nootropic effects, or therapeutic targets, is that it is reliant on the integration of these results into an existing framework of CNS function. For instance, hypothesizing that 5-HT_6 receptor antagonists may produce their nootropic effects by disinhibiting DGCs via their direct effects on mossy cells is only possible by interpreting our results within the context of an existing body of literature showing that mossy cells drive inhibition onto DGCs that gate information flow into the hippocampus (Scharfman, 1995; Hsu, 2007; Jinde et al., 2012). Furthermore, reverse-engineering complex therapies such as atypical antipsychotics or antidepressants was fruitful, in part, because the 5-HT_6 receptor had been sequenced and cloned the year before. These examples illustrate why a top-down guided approach is inextricably linked to, facilitated by, and can even blur distinction with, bottom-up mechanistically-driven studies elucidating basic CNS function. Thus, research aimed at novel therapy development needs to tackle this formidable endeavor via both approaches, letting the top-down approach "show us the way" and allowing the bottom-up approach lay the groundwork with which to understand and integrate this information.

Targeting Disinhibition To Treat Memory Deficits

A Validated and Pharmacologically Targetable Phenomenon for Memory Enhancement

Chapter 2 in this dissertation tested the effects of 5-HT_6 receptor ligands on inhibitory synaptic transmission in the DG. Results from these and other studies suggest that 5-HT_6 receptor antagonists exert their procognitive effects, in part, via a reduction in inhibition – a phenomenon also known as disinhibition. Defined as a transient and selective break in inhibition that alters the excitation-inhibition balance to promote excitability in postsynaptic targets, disinhibition was first explored for its role in basal ganglia function, particularly motor output and action selection (Goldberg et al., 2013). However, disinhibition is perhaps now best known for its role in learning and memory (Froemke, 2015; Letzkus et al., 2015). Because disinhibition is a fundamental gating mechanism hardwired into synapses, neurons, and circuits, there may be innumerable molecular, cellular, and network targets to implement disinhibition for therapeutic benefit.

There is a vast body of literature describing the complex molecular, cellular, network mechanisms orchestrating disinhibition in learning and memory (Froemke, 2015; Letzkus et al., 2015). As it may relate to 5-HT_6 receptors and other neuromodulatory systems, disinhibition is a reduction in inhibitory synaptic transmission that increases the probability that incoming excitatory synaptic events will sufficiently depolarize the postsynaptic neuron. This depolarization strengthens existing active inputs by relieving the magnesium block on NMDARs, and / or increasing the probability an input will achieve action potential threshold, and thus promote long-term synaptic plasticity. This

simplified example of disinhibition illustrates how a temporary lapse in inhibition can facilitate the survival, strengthening, and / or creation of new synapses, and thus, promote learning and memory.

Validation for disinhibition as a therapeutic target comes from studies pharmacologically attenuating inhibition for memory enhancement. For instance, administration of $GABA_A$ receptor antagonists targeting GABA receptors containing the alpha-5 subunit, which are found primarily in the hippocampus, have been shown to improve acquisition and recall during the hippocampal-dependent water maze task in healthy rodents (Collinson et al., 2002; Collinson et al., 2006). Moreover, blockade of $GABA_B$ receptors with selective antagonists has been shown to improve novel object and place recognition, as well as contextual fear learning in a transgenic model of Down's syndrome (Kleschevnikov et al., 2012). Together, these studies begin to establish disinhibition as a pharmacologically targetable neural process for the treatment of memory deficits in disease.

Although detailing the countless mechanisms and time-scales by which disinhibition occurs throughout the nervous system is beyond the scope of this discussion, neurotransmitters such as acetylcholine, dopamine, norepinephrine, oxytocin, and serotonin, have all been shown to regulate inhibition and are implicated in producing their effects, in part, via disinhibition (Froemke, 2015). These neurotransmitter systems influence behavior via a plethora of receptor subtypes and downstream signaling cascades that can be pharmacologically targeted by receptor subtype-specific ligands to disinhibit neural circuits and potentially treat cognitive dysfunction in disease. Together, this body of literature suggests disinhibition is a validated and pharmacologically

targetable mechanism for improving learning and memory and may be a general target for treating cognitive dysfunction in disease. However, there are several limitations to disinhibiting neural circuits for memory enhancement.

The Drawbacks and Dangers of Disinhibition

One limitation of targeting disinhibition to alleviate cognitive deficits in disease is disinhibition will likely be a symptomatic treatment that is unlikely to slow, halt, or prevent disease progression. Nevertheless, symptomatic treatments for memory deficits could ease suffering for millions of patients who currently have few or no treatments options available**,** delay admission into nursing homes, and vastly improve patients' quality of life (Gold and Budson, 2008; Raina et al., 2008; Gaugler et al., 2009). Therefore, the discovery of symptomatic treatments for cognitive dysfunction that act via disinhibition, or any mechanism, could dampen the looming financial and emotional burden of neurological disease and should be pursued alongside all other approaches to treating disease.

Another hurdle faced by treatments that attempt to implement disinhibition for the amelioration of memory deficits in disease is that disinhibition can disrupt the excitation-inhibition balance and possibly facilitate seizure activity (Staley, 2015). Particularly concerning is that many neurological disorders presenting with cognitive dysfunction also exhibit aberrant neuronal hyperexcitability and seizures. For instance, seizures are the primary symptom in epilepsy, and surveys indicate that epilepsy patients rate fear of another seizure and memory impairment amongst their greatest concerns (Thompson and Corcoran, 1992; Fisher et al., 2000). Thus, treating memory deficits in epilepsy patients

with treatments that facilitate excitatory network activity could exacerbate seizures, as well as aberrant epileptiform activity thought to disrupt memory function (Kleen et al., 2013). This risk also applies to patients with AD: Depending on the study, 1.5–64% of AD patients have had at least 1 unprovoked seizure (Friedman et al., 2012). Alarmingly, another study reported that half of all patients with AD and epilepsy have been shown to exhibit nonconvulsive "silent" seizures that may pass as amnestic spells or brief "blips" in consciousness (Vossel et al., 2013). Moreover, a longitudinal study in AD patients with no prior history of seizures reported that 42% of patients had epileptiform activity (compared to 10.5% of age-matched controls) (Vossel et al., 2016). Importantly, there were no differences in cognitive function between AD patients with and without epileptiform activity at the initial time of EEG testing. However, 3 years later, patients with epileptiform activity performed significantly worse on the Mini-Mental Status Exam, suggesting aberrant hyperexcitability is a risk factor for accelerated cognitive decline. Thus disinhibiting neural circuits to rescue memory impairment could worsen aberrant hyperexcitability and seizures in a manner that is difficult to recognize, and have long-term deleterious and counterproductive effects on cognition.

Disinhibiting Neural Circuits for Memory Enhancement: Precision Is Key

Assuming pharmacological treatments implementing disinhibition can be effective in patients without exacerbating the risk of seizures, there is yet another closely related formidable barrier: Globally increasing excitatory activity or attenuating inhibitory synaptic transmission is not sufficient. For example, attenuating inhibitory

synaptic transmission via optogenetics or overexpression of the gamma-aminobutyric acid transporter GAT1 can have deleterious effects on spatial memory formation (Hu et al., 2004; Andrews-Zwilling et al., 2012), suggesting that not all reductions in inhibition improve memory. Additionally, increasing inhibition onto DGCs via the addition of newly born neurons that drive inhibition in the DG has been shown to be necessary for normal cognitive function (Park et al., 2015). Thus, targeting disinhibition as a potential therapy requires spatially and temporally precise implementation that does not cause seizures (or other adverse effects such as motor impairment), and facilitates, rather than impairs memory formation. Towards this end, reverse-engineering treatments shown to exhibit procognitive and anticonvulsant effects such as 5-HT_6 receptor antagonists, which are thought to work via disinhibition, can provide a blueprint for how to properly and precisely implement disinhibition to maximize therapeutic benefits.

Cell Death and Destruction Diminish the Possibility of Therapeutic Benefit

Pharmacological treatments targeting disinhibition, or any treatment intended to ameliorate cognitive dysfunction, faces yet another barrier in neurological disease: Extensive brain damage increases the odds a patient will exhibit pharmacoresistance. For instance, by the time AD patients experience symptoms that substantially interfere with work and daily living, their brain is likely inundated with amyloid plaques and fibrillary tangles, and is undergoing temporal lobe and cortical atrophy (Frisoni et al., 2010). Unfortunately, AD patients with high levels of medial temporal lobe atrophy are less likely to respond to donepezil, the most commonly prescribed treatment for AD,

compared to patients with less damage (Connelly et al., 2005). Moreover, TLE patients exhibiting widespread neuron loss and hippocampal sclerosis (Thom, 2009; Thom et al., 2009) are more likely to be pharmacoresistant and cognitively impaired than those without noticeable damage (Helmstaedter, 2002). Together these studies highlight the idea that disinhibition, or any therapeutic target, may be difficult to implement in patients with overt cell loss. Moreover, promoting excitability via disinhibition of neural circuits may exacerbate cell loss via Ca^{2+}-dependent apoptotic mechanisms. However, not all hope is lost: Some neurons are quite resilient to cell death in disease. For instance, DGCs are fairly resistant to cell death in epilepsy, and not all hilar inhibitory interneurons and mossy cells die off (Wittner et al., 2001; Kobayashi and Buckmaster, 2003; Sun et al., 2007). Moreover, not all epilepsy patients demonstrating memory impairment have severe hippocampal damage (Aikia et al., 1995; Lah et al., 2014), and hippocampal cell loss is not always associated with memory impairment (Castro et al., 2013; Schmidt et al., 2015). Lastly, associations between hippocampal volume loss and performance on memory tests are highly variable in patients with epilepsy (Aikia et al., 2001). Together, these studies suggest that in some patients more subtle changes in hippocampal physiology may underlie memory dysfunction, and patients with overt cell death may still have essential neural circuitry in place that may be available for therapeutic benefit. Thus, targeting disinhibition may be useful in alleviating cognitive dysfunction, but will likely depend upon the degree of cell loss, brain damage, and remodeling.

In summary, as it relates to the work in this dissertation, a top-down guided approach attempts to elucidate an anatomical and physiological roadmap for treating diseases by reverse-engineering existing treatments that are not well understood but have

already been shown to alleviate symptoms in disease. Results from the present studies support a much larger body of work suggesting that disinhibition is a viable and pharmacologically targetable phenomenon for the symptomatic treatment of memory deficits. However, disinhibition must be precisely targeted, so as to not exacerbate the risk of seizures or cell death, while achieving efficacy in the presence of overt cell loss and remodeling that accompanies disease. Despite these formidable barriers, 5-HT_6 receptors (and other targets discussed below) offer proof of concept that procognitive and anticonvulsant therapies exist, and reverse-engineering their anatomical and physiological properties may provide a roadmap towards achieving procognitive and anticonvulsant therapies in patients with memory deficits and seizures.

Understanding Disease from the Bottom-Up:

The Pros and Cons

New Therapies and New Tools from Old Ways

Understanding the pathological basis of disease is a cornerstone of health science and is perhaps one of the greatest challenges researchers face. The enormity of this endeavor stems from the number of diseases, the innumerable dynamic and synergistic molecular, cellular, and network mechanisms that define their pathophysiology, and the complex heterogeneity underlying each disease. Despite these tremendous challenges, basic research into health and disease must continue, as it has led to, and will likely continue to yield new therapies.

For example, seminal studies identifying major neurotransmitter systems and their anatomical origins elucidated the underlying pathophysiology of many neurological

diseases. This pioneering work gave rise to our first approved treatments for AD and Parkinson's disease (PD) that are still is use today (Hornykiewicz, 2010; Contestabile, 2011). Identification and ablation of brainstem nuclei producing acetylcholine and dopamine revealed their role in higher cognitive function, and in movement and reward, respectively, prompting researchers to examine these brainstem nuclei in postmortem tissue from AD and PD patients. These foundational studies led to the development of treatments aimed at preventing the breakdown of acetylcholine, or facilitating the production of dopamine, giving rise to the first approved treatments for AD and PD that are still in use today – acetylcholinesterase inhibitors and L-Dopa. AD and PD are just 2 examples of how an understanding of the underlying etiology has contributed to the development of effective treatments for devastating neurological disorders. Patients with multiple sclerosis, schizophrenia, addiction, epilepsy, and countless other diseases outside the nervous system have also benefited from basic insights into biological function in health and disease.

In addition to providing insights into mechanisms driving disease, basic research bestows new technologies that catapult our understanding of biology. Undoubtedly, one of the latest advancements in neuroscience made possible by basic research has been optogenetics. Named "Breakthrough of the Decade" by *Science* in 2010 (News, 2010), incorporation of light-activation algal proteins into the mammalian genome has given researchers unprecedented spatial and temporal control over individual cell types, particularly the motley of inhibitory interneuron subtypes found throughout the brain, allowing scientists to probe their individual contribution to neurophysiology, and ultimately their effects on behavior. Currently underway, this revolution in neuroscience

would not be possible without a basic understanding of light-sensitive microbial opsins, as well as the genetics required to obtain their stable expression in the mammalian genome. Together, these examples provides insight into how basic research into neuroanatomy and neurotransmitter systems, as well as microorganism function, brought about our first treatments for AD and PD, and provides tools that revolutionize our understanding of the CNS.

Sorting Through the Complexity from the Bottom-Up

Advances in neurological science have reaped invaluable benefits from bottom-up mechanistic-driven studies into the fundamentals of nervous system function. However, the bottom-up approach faces perhaps the greatest challenge in all of biological science: Obtaining an understanding of the pathogenic mechanisms underlying disease. Insight into the mechanistic basis of disease continues to reveal increasing complexity perhaps best exemplified by AD. There are currently 5.3 millions Americans and 35 million people worldwide suffering from AD, with estimates projecting 100 million people will have AD by 2050 (Brookmeyer et al., 2007; Alzheimer's, 2015). And yet, the cause of sporadic AD, which makes up approximately 99% of cases, remain unknown. Numerous pathogenic mechanisms have been implicated in the etiology of AD including Aβ, accumulation of tau neurofibrillary tangles, autophagy dysfunction related to apolipoprotein E (APOE), hyperexcitability and seizures, oxidative stress and metabolic dysfunction, inflammation, neurovascular defects, and others, all of which have all been suspected to individually or synergistically contribute to the progressive loss of cognitive

function in AD (Huang and Mucke, 2012). Unfortunately, complex pathological heterogeneity is not unique to AD: Epilepsy is defined as a family of neurological disorders made up of varying pathophysiology that produce unprovoked spontaneous recurrent seizures. The causes of epilepsy have been attributed to single or multiple gene mutations, as well as environmental factors such as traumatic brain injury, brain tumors, stroke, infection, stress, and fevers. All of these events can trigger varying cellular, molecular, and network level cascades that contribute to seizures and associated comorbidities (Mody, 1998; Pitkanen and Lukasiuk, 2009, 2011; Goldberg and Coulter, 2013). The need to understand the underlying pathophysiology in AD and epilepsy is highlighted by the number of patients without treatment options. For example, 20–30% of patients with epilepsy do not have their seizures adequately controlled, despite the availability of over 20 FDA-approved ASDs (Sillanpaa and Schmidt, 2006; Brodie et al., 2012). Moreover, current treatments for cognitive dysfunction in AD are few in number, mechanistically homogenous, only marginally effective (Nygaard, 2013). However, the need to study the basic pathophysiology of disease is perhaps best highlighted by results from the latest clinical trials in AD, suggesting that targeting Aβ, commonly believed to be the primary pathophysiology thought to contribute to cognitive dysfunction in AD, has repeatedly failed (although targeting Aβ early in disease progression has shown some promise at delaying disease onset) (Loeffler, 2013; Nygaard, 2013). Together, these studies suggests that the etiology of AD is much more complex than was originally suspected, and the development of urgently needed treatments could be greatly facilitated by a mechanistic understanding of the complex pathophysiology in AD and epilepsy.

Hyperexcitability as a Therapeutic Target for Cognitive Dysfunction

Our studies into the mechanistic basis of memory deficits in corneal kindled mice revealed pathological hyperexcitability in DGCs and synaptic plasticity deficits at perforant-path DGC synapses in the absence of overt cell loss. These results fit into a much larger body of work reporting neuronal hyperexcitability, synaptic plasticity abnormalities, and cognitive impairment in other animal models of epilepsy thought to closely parallel findings in humans (Cossart et al., 2001; Hannesson et al., 2001; Sloviter et al., 2003; Harvey and Sloviter, 2005; Leung and Shen, 2006; Sloviter et al., 2006; Sun et al., 2007; Chauviere et al., 2009; Jinde et al., 2012). Our results differ from these models in that they were observed in a noninvasive readily producible model of corneal kindling that is not constrained by a laborious electrode implantation surgery and thus may have greater amenability for drug discovery and development. Although the causes of hyperexcitability and synaptic plasticity deficits in the DG of corneal kindled mice remain unknown, it is suspected that the observed attenuation in LTP in the DG may be a compensatory response to the over-activation of excitatory networks during kindling, which could have saturated the capability for further synaptic potentiation at the perforant-DGC synapse via metaplastic changes, as suggested by others (Huang et al., 1992; McIntyre et al., 2002; Meador, 2007). Regardless of the cause, studies suggest that treating seizures and pathological hyperexcitability with antiseizure drugs may not only reduce seizures, but may also alleviate cognitive dysfunction. This is not surprising, since hyperexcitability, as well as the seizures themselves, can impair cognition (Kleen et al., 2013; Holmes, 2015, 2016).

Treating hyperexcitability to alleviate seizures and cognitive dysfunction in epilepsy is perhaps best illustrated by the blockbuster ASD levetiracetam (LEV). LEV not only exhibits remarkable efficacy at reducing seizures, but also has a relatively benign side effect profile, particularly with respect to its effects on cognition. Remarkably, in a clinical trial, LEV not only reduced seizures in patients with AD, but patients also exhibited improved scores during tasks measuring attention and oral fluency (Cumbo and Ligori, 2010). Moreover, a neuroimaging study conducted in patients with mild cognitive impairment reported that LEV treatment attenuated pathological hyperexcitability in the hippocampus and improved patients' performance during a spatial memory test (Bakker et al., 2012). Thus, attenuating the underlying hyperexcitability in epilepsy or AD may restore proper cognitive function. Moreover, corneal kindled mice may be a novel model to explore the underlying causes of hyperexcitability in the absence of overt cell death, and a behavioral model for testing whether novel treatments that alleviate hyperexcitability rescue their cognitive impairments. However, targeting hyperexcitability to ease cognitive dysfunction is not without limitation.

One drawback to attenuating hyperexcitability for the treatment of seizures and cognitive dysfunction mirrors one of the difficulties with implementing disinhibition to treat cognitive dysfunction: Dampening neuronal excitability must be done precisely, or it can cause cognitive dysfunction, whereas disinhibiting neuronal circuits to improve memory must be done precisely to avoid cognitive dysfunction or seizures. These 2 are inextricably linked because they both affect the precise excitation-inhibition balance governing proper neural function. One example of treatments that imprecisely affect the

excitation-inhibition balance and cause cognitive dysfunction comes from ASDs: Carbamazepine, phenobarbital, phenytoin, topiramate, and valproate are all highly effective treatments for alleviating seizures, but have also been shown to impair memory and other aspects of cognitive function in healthy individuals, patients with epilepsy, and AD patients. (Meador et al., 1993; Meador et al., 1995; Lee et al., 2003; Meador et al., 2005; Salinsky et al., 2005; Cumbo and Ligori, 2010). Their beneficial effects on seizures and unwanted effects on cognition likely result from dampening excitatory network activity as evidenced by mild electroencephalogram (EEG) slowing (Meador et al., 1993). The reason for LEV's more benign effects on cognition are not well understood, however, LEV is thought to temper presynaptic vesicle release at excitatory synapses only during pathologically hyperexcitable conditions with excessive vesicle release, thus leaving vesicle release during normal levels of excitatory synaptic transmission unperturbed (Meehan et al., 2011). Nevertheless, these studies illustrate that treating hyperexcitability is an established pharmacologically targetable treatment option for seizures and cognitive dysfunction but must be done with precision to avoid unwanted side effects such as cognitive dysfunction itself. Moreover, it also reinforces the idea that reverse-engineering the effects of a drug such as LEV may be instructive in guiding future therapy development. Finally, these studies highlight the idea that treatments for cognitive dysfunction and seizures need not only improve memory or attenuate seizures; ideally, new treatments should be both procognitive and anticonvulsant.

The Future of Treating Cognitive Dysfunction in Disease: All Hope Is Not Lost

So far, I have highlighted the pros and cons of top-down and bottom-up guided approaches to new therapy development. Reverse-engineering existing treatments could pave the way to new therapeutic targets and perhaps highlight new network-level treatment strategies such as disinhibition to alleviate cognitive dysfunction in disease. On the other hand, bottom-up mechanistically driven studies have lead to disease-specific treatments in AD, PD, and other devastating neurological disorders and holds great promise for the development of disease-modifying therapies. Not only is each of these approaches invaluable, but they inform each other, such that a basic understanding of CNS function provides a framework for which to understand treatments already shown to alleviate disease symptoms, and that framework can be expanded and directed by mechanistic insight into existing treatments. In fact, the lines between these 2 strategies blur as they approach a final common goal: The development of new and improved therapies to treat disease.

Procognitive and Anticonvulsant Targets: A Double-Edged Sword

The results obtained in this dissertation and their overall implications on the treatment of cognitive dysfunction in disease present a double-edge sword. Investigation of 5-HT_6 receptors and other cognition enhancing manipulations of the CNS show that disinhibition may yield a rich source of therapeutic targets to treat cognitive dysfunction in disease. However, decades of literature suggest that hyperexcitability can cause

seizures and cognitive dysfunction, and dampening hyperexcitability may worsen cognitive function. These phenomena – disinhibition and hyperexcitability – intersect and give rise to the idea that treatments for cognitive dysfunction in disease should be both procognitive and anticonvulsant. Remarkably, several procognitive and anticonvulsant targets have emerged over the last 2 decades, providing encouragement that treatments with these seemingly paradoxical properties exist, and hope that mechanistic insights into procognitive and anticonvulsant targets may yield insights into appropriate ways to modulate neuronal function for therapeutic benefits in diseases presenting with cognitive dysfunction and seizures.

Given the increasing urgency for therapies that alleviate cognitive dysfunction in diseases comorbid with pathological hyperexcitability and seizures, procognitive and anticonvulsant targets hold tremendous therapeutic value. Research in Chapters 2 and 3 of this dissertation focused on 5-HT_6 receptor antagonists, which have been shown to improve cognitive function in rodents on a variety of tasks, and exhibit anticonvulsant effects during acute seizure testing and following pilocarpine treatment in rats. 5-HT_6 receptor antagonists are thought to achieve their procognitive effects via an attenuation of inhibitory synaptic transmission, and in accordance with their effects on synaptic transmission, results in our study suggest that they may exacerbate seizures depending on the species, strain, or type of seizure being tested. Although these results are preliminary and need to be confirmed, they are alarming because 5-HT_6 receptor antagonists are currently being tested in patients with AD and thus may be worsening nonconvulsive "silent" seizures or epileptiform activity that easily goes unnoticed. However, results in J20 mice suggest that 5-HT_6 receptor antagonists do not exacerbate seizures during the

minimal clonic seizure test in a model of AD. Nevertheless, further preclinical testing is needed to better understand how 5-HT_6 receptor antagonists affect different types of seizures. Clinical awareness of 5-HT_6 receptor antagonist effects on seizures and hyperexcitability in patients with AD is needed, as they may be having beneficial, negligible, or possibly deleterious effects on seizures.

Another promising procognitive and anticonvulsant target is the glycine transporter (GlyT1). GlyT1 inhibitors have been reported to increase seizure threshold in the maximal electroshock test, and suppress seizures following intrahippocampal kainate-induced epilepsy (Shen et al., 2015). Moreover, GlyT1 inhibitors have been shown to improve working memory and rescue performance deficits during the radial arm maze in an animal model of schizophrenia (Manahan-Vaughan et al., 2008; Singer et al., 2009). Interestingly, GlyT1 inhibitors are thought to have complex effects on synaptic transmission, having both excitatory and inhibitory effects via NMDA and glycine receptors, respectively. On the strength of the preclinical data, GlyT1 inhibitors have been evaluated for their effects on treating extrapyramidal symptoms in patients with schizophrenia. Although these studies had mixed results (Umbricht et al., 2014; Singer et al., 2015), to my knowledge, the effects of GlyT1 inhibitors on seizures and cognition in patients with epilepsy and AD have not been evaluated. In light of the preclinical demonstration of their procognitive and anticonvulsant effects, future studies should comprehensively test GlyT1 inhibitors for their procognitive and anticonvulsant effects in rodents, and attempt to reverse-engineer GlyT1 inhibitors' effects on CNS function to better understand their effects on seizures and cognition.

The histamine H_3 receptor (H_3R) is another target with procognitive and

anticonvulsant potential. Histamine is an endogenous anticonvulsant produced in the brain, and the H_3R receptor subtype is an autoreceptor that limits histamine release (Yokoyama et al., 1992; Brown et al., 2001). Despite the idea that histamine has endogenously inhibitory effects on neural circuits, blocking its release via H_3R receptor antagonists exhibits anticonvulsant activity in rats during the maximal electroshock test (Sadek et al., 2016). Moreover, the H_3R antagonist Pitolisant exhibited anticonvulsant activity in an early Phase II study in humans with photosensitive epilepsy by attenuating photoparoxysmal response monitored via EEG (Kasteleijn-Nolst Trenite et al., 2013). These results warrant larger Phase II clinical studies evaluating the effects of H_3R antagonists on seizures. In addition to their preclinical and clinical anticonvulsant effects, treatment with an H_3R antagonist or inverse agonist have been shown to improve memory consolidation, retrieval, and retention during a 1-trial inhibitory avoidance task in healthy and scopolamine-treated rodents (Charlier et al., 2013; Sadek et al., 2016). Interestingly, H_3R antagonists are thought to increase levels of acetylcholine, just like 5-HT_6 receptor antagonists, but differ in that they have been shown to *increase* the release of GABA, whereas 5-HT_6 receptors antagonists are thought to *decrease* the release of GABA (Yamamoto et al., 1997; Haas et al., 2008). Thus, future studies that disentangle the complex effects of histamine and H_3R blockade on brain function could provide novel insights into regulating neural circuits for procognitive and anticonvulsant effects that differ from 5-HT_6 receptors.

Together, these 3 targets, 5-HT_6, GlyT1, and H_3R validate that procognitive and anticonvulsant treatment targets exist, are pharmacologically targetable, and may achieve similar therapeutic endpoints via differing effects on synaptic transmission. Moreover,

reverse-engineering their effects on brain function may elucidate the mechanisms by which these targets achieve their procognitive and anticonvulsant effects, and potentially highlight new therapeutic approaches or targets to effectively and simultaneously treat cognitive function and seizures in disease.

A Polytherapeutic Approach to Treating Cognitive Dysfunction in Disease

Given the complex underlying etiology of cognitive dysfunction in AD, it is unlikely that any one drug therapy or "magic bullet" is going to prevent and / or rescue cognitive dysfunction in AD. Rather, it is likely that a polytherapeutic approach will need to be employed. Classically defined by the accumulation of Aβ plaques, AD is now thought to arise from a much more complex and diverse set of pathophysiological mechanisms that is not well understood (Huang and Mucke, 2012; Nygaard, 2013). The multifactorial pathophysiology underlying AD is further highlighted by mixed, but mostly failed, results of clinical trials targeting Aβ (Yang and Xiao, 2015; Sevigny et al., 2016), as well as studies showing that cognitive dysfunction can be rescued in animal models and patients with AD by treating hyperexcitability without reducing the pathological accumulation of Aβ (Cumbo and Ligori, 2010; Bakker et al., 2012; Sanchez et al., 2012). Although treatments targeting Aβ may still be effective and necessary to halt disease progression, successful treatment of AD will likely require early identification of individuals who will develop AD long before symptoms have arisen, a polytherapeutic approach to prevent one or several of the underlying pathologies from progressing, and symptomatic treatment with cognition enhancing drugs.

Putting Neurons Back: Are Stem Cells the Future of Treating Cognitive Dysfunction in Neurodegenerative Disorders?

Until treatments preventing cell loss and destruction in AD become available, the latest hope on the horizon for neurodegenerative disorders is stem-cell therapy. Although still in its infancy, major breakthroughs in the last decade have established the possibility of stem-cell therapy for replacing cells lost in disease. For instance, bilateral implantation of GABAergic progenitor cells in the hippocampi of mice with pilocarpine-induced epilepsy produced complete remediation of spontaneous seizures and memory dysfunction during Morris water maze and novel object recognition tasks (Hunt et al., 2013). Moreover, stem cell therapy has been shown to rescue cognitive function during working memory and novel place recognition tasks in 2 rodent models of AD (Wang et al., 2006; Yamasaki et al., 2007). Additionally, the possibility of treating patients with stem cell therapy recently overcame a major hurdle, as reprograming of patient-derived somatic stem cells may circumvent immune rejection (Tincer et al., 2016). Moreover, these advances may facilitate personalized medicine by using patient-derived cells for preclinical drug screening, and also guide patient selection for clinical trials targeting a particular AD-related pathophysiology (Israel et al., 2012). Although the translation of stem cells into patient therapies is still greatly hampered by the threat of tumor generation, stem cell therapy holds great promise for the treatment of intractable neurological disorders, particularly those accompanied by overt cell loss (Dantuma et al., 2010). Additionally, the possibility of stem cell therapy and personalized medicine provide an excellent example of how basic research – in this case, programming and

reprogramming progenitors cells – can lead to the development of new therapies and potentially revolutionize how we treat disease.

Cognitive Enhancement in Healthy Individuals: Social and Ethical Implications

There is clearly a need to develop therapies to alleviate memory deficits in disease. However, the development of procognitive therapies such as amphetamine and methylphenidate has already led to their availability and use in healthy individuals as cognitive enhancers. Their use outside of disease would be expected to increase with the development of new cognition enhancing drugs, which has a number of implications and limitations. For instance, improving cognitive function in healthy individuals could have numerous societal benefits: Scientists, clinicians, engineers, mathematicians, military and other safety personnel, as well as construction and transportation workers, could all potentially achieve greater productivity and safety aided by cognitive enhancement. However, these benefits are not without constraint. Ethical considerations need to be taken, as limited availability of cognitive enhancement to those who can and cannot afford them may create a socioeconomic divide (Mehlman, 2004). Moreover, the long-term effects of cognitive enhancement need to be evaluated before individuals begin their long-term use, as these compounds could have deleterious health effects. Nevertheless, researchers are in hot pursuit of cognition enhancing compounds and it is likely that new and improved nootropics will eventually become commercially available.

Future Directions and Closing Remarks

Future studies attempting to better understand how 5-HT_6 receptors affect brain function could conduct a similar set of experiments performed in Chapter 2 in brain slices prepared from animals expressing genetically encoded calcium indicators. This would allow changes in neuronal excitability to be visualized and quantified in several mossy cells simultaneously without having to directly and individually record from them. Additionally, ongoing experiments are testing the hypothesis that mossy cells mediate the observed effects of 5-HT_6 receptor ligands on inhibitory synaptic transmission by repeating experiments performed in Chapter 2 in the presence of drugs that block glutamatergic synaptic transmission. As a follow up to Chapters 3 and 4, the effects of 5-HT_6 receptor antagonists on cognitive function and seizures should be comprehensively tested in multiple models of epilepsy, such as kainate- or pilocarpine-induced epilepsy, or perhaps in corneal kindled C57BL/6 mice. It would also be interesting to test the effects of 5-HT_6 receptor antagonists on cognitive function in combination with other ASDs shown to have more favorable effects on cognition such as LEV. Beneficial effects observed during combinatorial drug studies with LEV would facilitate the advancement of 5-HT_6 receptor antagonists into clinical trials for treating cognitive dysfunction in epilepsy and encourage future efforts to better understand how 5-HT_6 receptors affect brain function. Lastly, future studies should further evaluate the procognitive and anticonvulsant properties of GlyT1 inhibitors and H_3R inverse agonists in models of epilepsy and AD, either alone, or in combination with other ASDs.

In summary, studies attempting to reverse-engineer existing treatments or elucidate the mechanistic basis of disease have advanced our understanding and treatment

of cognitive dysfunction in disease. Neither of these approaches should be neglected nor favored; rather, both should be implemented simultaneously to maximize benefit. Results from the present studies suggest targeting disinhibition and hyperexcitability for the treatment of memory deficits. However, these broad therapeutic targets overlap and highlight the idea that treatments for cognitive dysfunction in disease need to be both procognitive and anticonvulsant. A number of receptor targets including 5-HT_6 receptors, H_3R, and GlyT1 suggest pharmacologically targetable procognitive and anticonvulsant treatments exist, and future studies untangling their complex effects on brain function could provide insights that facilitate the development of additional procognitive and anticonvulsant therapies. Lastly, symptomatic treatments for memory impairments will likely be part of a larger polytherapeutic armamentarium that also targets the underlying pathophysiology to slow or halt disease progression, and possibly reverse disease symptoms via stem-cell therapy. Together, both top-down and bottom-up guided approaches hold great promise in directing new therapy development for the treatment of memory deficits in disease.

References

Aikia M, Kalviainen R, Riekkinen PJ (1995) Verbal learning and memory in newly diagnosed partial epilepsy. Epilepsy Res 22:157-164.

Aikia M, Salmenpera T, Partanen K, Kalviainen R (2001) Verbal memory in newly diagnosed patients and patients with chronic left temporal lobe epilepsy. Epilepsy Behav 2:20-27.

Alzheimer's A (2015) 2015 Alzheimer's disease facts and figures. Alzheimers Dement 11:332-384.

Andrews-Zwilling Y, Gillespie AK, Kravitz AV, Nelson AB, Devidze N, Lo I, Yoon SY, Bien-Ly N, Ring K, Zwilling D, Potter GB, Rubenstein JL, Kreitzer AC, Huang Y (2012) Hilar GABAergic interneuron activity controls spatial learning and memory retrieval. PLoS One 7:e40555.

Bakker A, Krauss GL, Albert MS, Speck CL, Jones LR, Stark CE, Yassa MA, Bassett SS, Shelton AL, Gallagher M (2012) Reduction of hippocampal hyperactivity improves cognition in amnestic mild cognitive impairment. Neuron 74:467-474.

Brodie MJ, Barry SJ, Bamagous GA, Norrie JD, Kwan P (2012) Patterns of treatment response in newly diagnosed epilepsy. Neurology 78:1548-1554.

Brookmeyer R, Johnson E, Ziegler-Graham K, Arrighi HM (2007) Forecasting the global burden of Alzheimer's disease. Alzheimers Dement 3:186-191.

Brown RE, Stevens DR, Haas HL (2001) The physiology of brain histamine. Prog Neurobiol 63:637-672.

Castro LH, Silva LC, Adda CC, Banaskiwitz NH, Xavier AB, Jorge CL, Valerio RM, Nitrini R (2013) Low prevalence but high specificity of material-specific memory impairment in epilepsy associated with hippocampal sclerosis. Epilepsia 54:1735-1742.

Charlier Y, Brabant C, Serrano ME, Lamberty Y, Tirelli E (2013) The prototypical histamine H3 receptor inverse agonist thioperamide improves multiple aspects of memory processing in an inhibitory avoidance task. Behav Brain Res 253:121-127.

Chauviere L, Rafrafi N, Thinus-Blanc C, Bartolomei F, Esclapez M, Bernard C (2009) Early deficits in spatial memory and theta rhythm in experimental temporal lobe epilepsy. J Neurosci 29:5402-5410.

Codony X, Vela JM, Ramirez MJ (2011) 5-HT(6) receptor and cognition. Curr Opin Pharmacol 11:94-100.

Collinson N, Atack JR, Laughton P, Dawson GR, Stephens DN (2006) An inverse agonist selective for alpha5 subunit-containing GABAA receptors improves encoding

and recall but not consolidation in the Morris water maze. Psychopharmacology (Berl) 188:619-628.

Collinson N, Kuenzi FM, Jarolimek W, Maubach KA, Cothliff R, Sur C, Smith A, Otu FM, Howell O, Atack JR, McKernan RM, Seabrook GR, Dawson GR, Whiting PJ, Rosahl TW (2002) Enhanced learning and memory and altered GABAergic synaptic transmission in mice lacking the alpha 5 subunit of the GABAA receptor. J Neurosci 22:5572-5580.

Connelly PJ, Prentice NP, Fowler KG (2005) Predicting the outcome of cholinesterase inhibitor treatment in Alzheimer's disease. J Neurol Neurosurg Psychiatry 76:320-324.

Contestabile A (2011) The history of the cholinergic hypothesis. Behav Brain Res 221:334-340.

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, De Felipe J, Ben-Ari Y, Esclapez M, Bernard C (2001) Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nat Neurosci 4:52-62.

Cumbo E, Ligori LD (2010) Levetiracetam, lamotrigine, and phenobarbital in patients with epileptic seizures and Alzheimer's disease. Epilepsy Behav 17:461-466.

Dantuma E, Merchant S, Sugaya K (2010) Stem cells for the treatment of neurodegenerative diseases. Stem Cell Res Ther 1:37.

Dawson LA, Nguyen HQ, Li P (2001) The 5-HT(6) receptor antagonist SB-271046 selectively enhances excitatory neurotransmission in the rat frontal cortex and hippocampus. Neuropsychopharmacology 25:662-668.

Fisher RS, Vickrey BG, Gibson P, Hermann B, Penovich P, Scherer A, Walker S (2000) The impact of epilepsy from the patient's perspective I. Descriptions and subjective perceptions. Epilepsy Res 41:39-51.

Fone KC (2008) An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. Neuropharmacology 55:1015-1022.

Friedman D, Honig LS, Scarmeas N (2012) Seizures and epilepsy in Alzheimer's disease. CNS Neurosci Ther 18:285-294.

Frisoni GB, Fox NC, Jack CR, Jr., Scheltens P, Thompson PM (2010) The clinical use of structural MRI in Alzheimer disease. Nat Rev Neurol 6:67-77.

Froemke RC (2015) Plasticity of cortical excitatory-inhibitory balance. Annu Rev Neurosci 38:195-219.

Gaugler JE, Yu F, Krichbaum K, Wyman JF (2009) Predictors of nursing home admission for persons with dementia. Med Care 47:191-198.

Gold CA, Budson AE (2008) Memory loss in Alzheimer's disease: implications for development of therapeutics. Expert Rev Neurother 8:1879-1891.

Goldberg EM, Coulter DA (2013) Mechanisms of epileptogenesis: a convergence on neural circuit dysfunction. Nat Rev Neurosci 14:337-349.

Goldberg JH, Farries MA, Fee MS (2013) Basal ganglia output to the thalamus: still a paradox. Trends Neurosci 36:695-705.

Haas HL, Sergeeva OA, Selbach O (2008) Histamine in the nervous system. Physiol Rev 88:1183-1241.

Hannesson DK, Mohapel P, Corcoran ME (2001) Dorsal hippocampal kindling selectively impairs spatial learning/short-term memory. Hippocampus 11:275-286.

Harvey BD, Sloviter RS (2005) Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. J Comp Neurol 488:442-463.

Heal DJ, Smith SL, Fisas A, Codony X, Buschmann H (2008) Selective 5-HT6 receptor ligands: progress in the development of a novel pharmacological approach to the treatment of obesity and related metabolic disorders. Pharmacol Ther 117:207-231.

Helmstaedter C (2002) Effects of chronic epilepsy on declarative memory systems. Prog Brain Res 135:439-453.

Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM, Riley G, Smith DR, Bartlett S, Heidbreder CA, Atkins AR, Lacroix LP, Dawson LA, Foley AG, Regan CM, Upton N (2006) SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. Eur J Pharmacol 553:109-119.

Holmes GL (2015) Cognitive impairment in epilepsy: the role of network abnormalities. Epileptic Disord 17:101-116.

Holmes GL (2016) Effect of seizures on the developing brain and cognition. Semin Pediatr Neurol 23:120-126.

Hornykiewicz O (2010) A brief history of levodopa. J Neurol 257:S249-252.

Hsu D (2007) The dentate gyrus as a filter or gate: a look back and a look ahead. Prog Brain Res 163:601-613.

Hu JH, Ma YH, Jiang J, Yang N, Duan SH, Jiang ZH, Mei ZT, Fei J, Guo LH (2004) Cognitive impairment in mice over-expressing gamma-aminobutyric acid transporter 1 (GAT1). Neuroreport 15:9-12.

Huang Y, Mucke L (2012) Alzheimer mechanisms and therapeutic strategies. Cell

148:1204-1222.

Huang YY, Colino A, Selig DK, Malenka RC (1992) The influence of prior synaptic activity on the induction of long-term potentiation. Science 255:730-733.

Hunt RF, Girskis KM, Rubenstein JL, Alvarez-Buylla A, Baraban SC (2013) GABA progenitors grafted into the adult epileptic brain control seizures and abnormal behavior. Nat Neurosci 16:692-697.

Israel MA, Yuan SH, Bardy C, Reyna SM, Mu Y, Herrera C, Hefferan MP, Van Gorp S, Nazor KL, Boscolo FS, Carson CT, Laurent LC, Marsala M, Gage FH, Remes AM, Koo EH, Goldstein LS (2012) Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. Nature 482:216-220.

Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K (2012) Hilar mossy cell degeneration causes transient dentate granule cell hyperexcitability and impaired pattern separation. Neuron 76:1189-1200.

Kasteleijn-Nolst Trenite D, Parain D, Genton P, Masnou P, Schwartz JC, Hirsch E (2013) Efficacy of the histamine 3 receptor (H3R) antagonist pitolisant (formerly known as tiprolisant; BF2.649) in epilepsy: dose-dependent effects in the human photosensitivity model. Epilepsy Behav 28:66-70.

Kleen JK, Scott RC, Holmes GL, Roberts DW, Rundle MM, Testorf M, Lenck-Santini PP, Jobst BC (2013) Hippocampal interictal epileptiform activity disrupts cognition in humans. Neurology 81:18-24.

Kleschevnikov AM, Belichenko PV, Faizi M, Jacobs LF, Htun K, Shamloo M, Mobley WC (2012) Deficits in cognition and synaptic plasticity in a mouse model of Down syndrome ameliorated by GABAB receptor antagonists. J Neurosci 32:9217-9227.

Kobayashi M, Buckmaster PS (2003) Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. J Neurosci 23:2440-2452.

Lah S, Mohamed A, Thayer Z, Miller L, Diamond K (2014) Accelerated long-term forgetting of verbal information in unilateral temporal lobe epilepsy: Is it related to structural hippocampal abnormalities and/or incomplete learning? J Clin Exp Neuropsychol 36:158-169.

Lee S, Sziklas V, Andermann F, Farnham S, Risse G, Gustafson M, Gates J, Penovich P, Al-Asmi A, Dubeau F, Jones-Gotman M (2003) The effects of adjunctive topiramate on cognitive function in patients with epilepsy. Epilepsia 44:339-347.

Letzkus JJ, Wolff SB, Luthi A (2015) Disinhibition, a circuit mechanism for associative learning and memory. Neuron 88:264-276.

Leung LS, Shen B (2006) Hippocampal CA1 kindling but not long-term potentiation disrupts spatial memory performance. Learn Mem 13:18-26.

Loeffler DA (2013) Intravenous immunoglobulin and Alzheimer's disease: what now? J Neuroinflammation 10:70.

Maher-Edwards G, Dixon R, Hunter J, Gold M, Hopton G, Jacobs G, Hunter J, Williams P (2011) SB-742457 and donepezil in Alzheimer disease: a randomized, placebo-controlled study. Int J Geriatr Psychiatry 26:536-544.

Maher-Edwards G, Zvartau-Hind M, Hunter AJ, Gold M, Hopton G, Jacobs G, Davy M, Williams P (2010) Double-blind, controlled phase II study of a 5-HT6 receptor antagonist, SB-742457, in Alzheimer's disease. Curr Alzheimer Res 7:374-385.

Manahan-Vaughan D, Wildforster V, Thomsen C (2008) Rescue of hippocampal LTP and learning deficits in a rat model of psychosis by inhibition of glycine transporter-1 (GlyT1). Eur J Neurosci 28:1342-1350.

McIntyre DC, Poulter MO, Gilby K (2002) Kindling: some old and some new. Epilepsy Res 50:79-92.

Meador KJ (2007) The basic science of memory as it applies to epilepsy. Epilepsia 48 Suppl 9:23-25.

Meador KJ, Loring DW, Abney OL, Allen ME, Moore EE, Zamrini EY, King DW (1993) Effects of carbamazepine and phenytoin on EEG and memory in healthy adults. Epilepsia 34:153-157.

Meador KJ, Loring DW, Moore EE, Thompson WO, Nichols ME, Oberzan RE, Durkin MW, Gallagher BB, King DW (1995) Comparative cognitive effects of phenobarbital, phenytoin, and valproate in healthy adults. Neurology 45:1494-1499.

Meador KJ, Loring DW, Vahle VJ, Ray PG, Werz MA, Fessler AJ, Ogrocki P, Schoenberg MR, Miller JM, Kustra RP (2005) Cognitive and behavioral effects of lamotrigine and topiramate in healthy volunteers. Neurology 64:2108-2114.

Meehan AL, Yang X, McAdams BD, Yuan L, Rothman SM (2011) A new mechanism for antiepileptic drug action: vesicular entry may mediate the effects of levetiracetam. J Neurophysiol 106:1227-1239.

Mehlman MJ (2004) Cognition-enhancing drugs. Milbank Q 82:483-506.

Mody I (1998) Ion channels in epilepsy. Int Rev Neurobiol 42:199-226.

News S (2010) Insights of the decade. Stepping away from the trees for a look at the forest. Introduction. Science 330:1612-1613.

Nygaard HB (2013) Current and emerging therapies for Alzheimer's disease. Clin Ther 35:1480-1489.

Park EH, Burghardt NS, Dvorak D, Hen R, Fenton AA (2015) Experience-dependent

regulation of dentate gyrus excitability by adult-born granule cells. J Neurosci 35:11656-11666.

Pitkanen A, Lukasiuk K (2009) Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. Epilepsy Behav 14 Suppl 1:16-25.

Pitkanen A, Lukasiuk K (2011) Mechanisms of epileptogenesis and potential treatment targets. Lancet Neurol 10:173-186.

Raina P, Santaguida P, Ismaila A, Patterson C, Cowan D, Levine M, Booker L, Oremus M (2008) Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. Ann Intern Med 148:379-397.

Ramirez MJ (2013) 5-HT6 receptors and Alzheimer's disease. Alzheimers Res Ther 5:15.

Roth BL, Craigo SC, Choudhary MS, Uluer A, Monsma FJ, Jr., Shen Y, Meltzer HY, Sibley DR (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J Pharmacol Exp Ther 268:1403-1410.

Sadek B, Saad A, Subramanian D, Shafiullah M, Lazewska D, Kiec-Kononowiczc K (2016) Anticonvulsant and procognitive properties of the non-imidazole histamine H3 receptor antagonist DL77 in male adult rats. Neuropharmacology 106:46-55.

Salinsky MC, Storzbach D, Spencer DC, Oken BS, Landry T, Dodrill CB (2005) Effects of topiramate and gabapentin on cognitive abilities in healthy volunteers. Neurology 64:792-798.

Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu GQ, Palop JJ, Mucke L (2012) Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc Natl Acad Sci U S A 109:E2895-2903.

Scharfman HE (1995) Electrophysiological evidence that dentate hilar mossy cells are excitatory and innervate both granule cells and interneurons. J Neurophysiol 74:179-194.

Schechter LE, Lin Q, Smith DL, Zhang G, Shan Q, Platt B, Brandt MR, Dawson LA, Cole D, Bernotas R, Robichaud A, Rosenzweig-Lipson S, Beyer CE (2008) Neuropharmacological profile of novel and selective 5-HT6 receptor agonists: WAY-181187 and WAY-208466. Neuropsychopharmacology 33:1323-1335.

Schmidt CS, Lassonde M, Gagnon L, Sauerwein CH, Carmant L, Major P, Paquette N, Lepore F, Gallagher A (2015) Neuropsychological functioning in children with temporal lobe epilepsy and hippocampal atrophy without mesial temporal sclerosis: a distinct clinical entity? Epilepsy Behav 44:17-22.

Sevigny J, Chiao P, Bussiere T, Weinreb PH, Williams L, Maier M, Dunstan R, Salloway S, Chen T, Ling Y, O'Gorman J, Qian F, Arastu M, Li M, Chollate S, Brennan MS, Quintero-Monzon O, Scannevin RH, Arnold HM, Engber T, Rhodes K, Ferrero J, Hang Y, Mikulskis A, Grimm J, Hock C, Nitsch RM, Sandrock A (2016) The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. Nature 537:50-56.

Shen HY, van Vliet EA, Bright KA, Hanthorn M, Lytle NK, Gorter J, Aronica E, Boison D (2015) Glycine transporter 1 is a target for the treatment of epilepsy. Neuropharmacology 99:554-565.

Sillanpaa M, Schmidt D (2006) Natural history of treated childhood-onset epilepsy: prospective, long-term population-based study. Brain 129:617-624.

Singer P, Dubroqua S, Yee BK (2015) Inhibition of glycine transporter 1: The yellow brick road to new schizophrenia therapy? Curr Pharm Des 21:3771-3787.

Singer P, Feldon J, Yee BK (2009) The glycine transporter 1 inhibitor SSR504734 enhances working memory performance in a continuous delayed alternation task in C57BL/6 mice. Psychopharmacology (Berl) 202:371-384.

Sloviter RS, Zappone CA, Harvey BD, Bumanglag AV, Bender RA, Frotscher M (2003) "Dormant basket cell" hypothesis revisited: relative vulnerabilities of dentate gyrus mossy cells and inhibitory interneurons after hippocampal status epilepticus in the rat. J Comp Neurol 459:44-76.

Sloviter RS, Zappone CA, Harvey BD, Frotscher M (2006) Kainic acid-induced recurrent mossy fiber innervation of dentate gyrus inhibitory interneurons: possible anatomical substrate of granule cell hyper-inhibition in chronically epileptic rats. J Comp Neurol 494:944-960.

Staley K (2015) Molecular mechanisms of epilepsy. Nat Neurosci 18:367-372.

Sun C, Mtchedlishvili Z, Bertram EH, Erisir A, Kapur J (2007) Selective loss of dentate hilar interneurons contributes to reduced synaptic inhibition of granule cells in an electrical stimulation-based animal model of temporal lobe epilepsy. J Comp Neurol 500:876-893.

Thom M (2009) Hippocampal sclerosis: progress since Sommer. Brain Pathol 19:565-572.

Thom M, Martinian L, Catarino C, Yogarajah M, Koepp MJ, Caboclo L, Sisodiya SM (2009) Bilateral reorganization of the dentate gyrus in hippocampal sclerosis: a postmortem study. Neurology 73:1033-1040.

Thompson PJ, Corcoran R (1992) Everyday memory failures in people with epilepsy. Epilepsia 33 Suppl 6:S18-20.

Tincer G, Mashkaryan V, Bhattarai P, Kizil C (2016) Neural stem/progenitor cells in Alzheimer's disease. Yale J Biol Med 89:23-35.

Umbricht D, Alberati D, Martin-Facklam M, Borroni E, Youssef EA, Ostland M, Wallace TL, Knoflach F, Dorflinger E, Wettstein JG, Bausch A, Garibaldi G, Santarelli L (2014) Effect of bitopertin, a glycine reuptake inhibitor, on negative symptoms of schizophrenia: a randomized, double-blind, proof-of-concept study. JAMA Psychiatry 71:637-646.

Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, Hegde M, Cornes SB, Henry ML, Nelson AB, Seeley WW, Geschwind MD, Gorno-Tempini ML, Shih T, Kirsch HE, Garcia PA, Miller BL, Mucke L (2013) Seizures and epileptiform activity in the early stages of Alzheimer disease. JAMA Neurol 70:1158-1166.

Vossel KA, Ranasinghe KG, Beagle AJ, Mizuiri D, Honma SM, Dowling AF, Darwish SM, Van Berlo V, Barnes DE, Mantle M, Karydas AM, Coppola G, Roberson ED, Miller BL, Garcia PA, Kirsch HE, Mucke L, Nagarajan SS (2016) Incidence and impact of subclinical epileptiform activity in Alzheimer's disease. Ann Neurol 80:858-870.

Wang Q, Matsumoto Y, Shindo T, Miyake K, Shindo A, Kawanishi M, Kawai N, Tamiya T, Nagao S (2006) Neural stem cells transplantation in cortex in a mouse model of Alzheimer's disease. J Med Invest 53:61-69.

Wilkinson D, Windfeld K, Colding-Jorgensen E (2014) Safety and efficacy of idalopirdine, a 5-HT6 receptor antagonist, in patients with moderate Alzheimer's disease (LADDER): a randomised, double-blind, placebo-controlled phase 2 trial. Lancet Neurol 13:1092-1099.

Wittner L, Magloczky Z, Borhegyi Z, Halasz P, Toth S, Eross L, Szabo Z, Freund TF (2001) Preservation of perisomatic inhibitory input of granule cells in the epileptic human dentate gyrus. Neuroscience 108:587-600.

Woolley ML, Marsden CA, Fone KC (2004) 5-ht6 receptors. Curr Drug Targets CNS Neurol Disord 3:59-79.

Yamamoto Y, Mochizuki T, Okakura-Mochizuki K, Uno A, Yamatodani A (1997) Thioperamide, a histamine H3 receptor antagonist, increases GABA release from the rat hypothalamus. Methods Find Exp Clin Pharmacol 19:289-298.

Yamasaki TR, Blurton-Jones M, Morrissette DA, Kitazawa M, Oddo S, LaFerla FM (2007) Neural stem cells improve memory in an inducible mouse model of neuronal loss. J Neurosci 27:11925-11933.

Yang C, Xiao S (2015) New developments of clinical trial in immunotherapy for Alzheimer's disease. Curr Pharm Biotechnol 16:484-491.

Yokoyama H, Onodera K, Maeyama K, Yanai K, Iinuma K, Tuomisto L, Watanabe T

(1992) Histamine levels and clonic convulsions of electrically-induced seizure in mice: the effects of alpha-fluoromethylhistidine and metoprine. Naunyn Schmiedebergs Arch Pharmacol 346:40-45.

Yun HM, Rhim H (2011) The serotonin-6 receptor as a novel therapeutic target. Exp Neurobiol 20:159-168.

APPENDIX

DETECTING THE EFFECTS OF INHIBITORY SYNAPTIC TRANSMISSION ON LONG-TERM POTENTIATION IN THE DENTATE GYRUS *IN VITRO*

Abstract

The goal of this study was to test the effects of a 5-HT_6 receptor antagonist on theta-burst stimulation- (TBS) induced long-term potentiation (LTP) in the dentate gyrus (DG) in brain slices prepared from a transgenic mouse model of Alzheimer's disease (AD). Inducing robust LTP in the DG *in vitro* using theta-burst stimulation (TBS) requires bath application of drugs that block inhibitory synaptic transmission but precludes experimentation with the effects of inhibition on LTP. This was particularly problematic since 5-HT_6 receptors were shown to bidirectionally modulate inhibition in the DG in Chapter 2, and J20 mice, a transgenic model of AD, exhibit pathologically increased inhibition in the DG. To circumvent complete blockade of inhibition, we established a reliable TBS LTP induction paradigm in the presence of 50 μM picrotoxin (PTX) (2 trains of 4 pulses at 100 Hz separated by 200 ms, repeated 5 times with a 20 s interval). Next, a concentration-response study with PTX revealed an EC_{50} of 4 μM. We then found that the $GABA_A$ receptor agonist diazepam (50 μM) attenuated LTP in 4 μM PTX, suggesting these conditions are sensitive to increased inhibition. Next, we tested the

5-HT_6 receptor antagonist SB-399885 (1 μM) in slices prepared from J20 mice and their nontransgenic (NTG) littermates: LTP in J20 and NTG littermates did not differ, and SB-399885 had no effect on LTP in either genotype. At this time, the 4-channel Slicemaster was updated to an 8-channel Slicemaster, and our TBS LTP induction paradigm was no longer attenuated by 50 μM DZP. We tested several LTP induction paradigms, none of which were sensitive to DZP. Lastly, we retested our initial TBS LTP induction paradigm in the presence of 4 and 50 μM PTX in J20 mice: LTP in J20 mice was not altered in either condition, although results in 50 μM PTX may have been underpowered. Nevertheless, establishing an *in vitro* TBS LTP induction paradigm in the DG that is sensitive to increased inhibition is possible and may be useful for detecting the effects of drugs that regulate LTP in the DG via inhibitory synaptic transmission.

Introduction

The first goal of this study was to establish an *in vitro* theta-burst stimulation (TBS) long-term potentiation (LTP) induction paradigm that reliably produced LTP in the dentate gyrus (DG). We also wanted a reliable a TBS LTP induction paradigm that is sensitive to changes in GABAergic synaptic transmission. These objectives stem from our ultimate goal of this research, which was to test the effects of a 5-HT_6 receptor antagonist on LTP in a transgenic model of Alzheimer's disease (AD) that exhibits GABAergic remodeling in the DG. These data were intended to support other studies conducted in this dissertation, particularly our evaluation of 5-HT_6 receptor antagonists in J20 mice during a DG-associated spatial pattern processing paradigm, the metric task, in Chapter 3.

Prior to the experiments in this Appendix, we published a study testing the effects of the anticonvulsant drugs on LTP in the DG (West et al., 2014). Using a TBS paradigm that is routinely used by our lab to induce LTP in hippocampal area CA1, we found that the anticonvulsant valproic acid (VPA) attenuated LTP in the DG. However, the CA1 TBS LTP induction paradigm did not reliably produce LTP when adopted for use in the DG and thus, required testing in many brain slices to complete the experiment for publication. Therefore, we wanted to establish a TBS-LTP induction paradigm that more reliably produced LTP at perforant-path DG granule cell (DGC) synapses.

Besides reliability, we also wanted a DG TBS LTP induction paradigm sensitive to alterations in GABAergic synaptic transmission, which presents an experimental conundrum. LTP at perforant path-DGC synapses can be produced *in vitro* using high-frequency stimulation (HFS) or TBS. However, HFS-induced LTP at perforant path DG synapses lacks sensitivity to inhibitory synaptic transmission (Chapman et al., 1998; Arima-Yoshida et al., 2011), and is considered a nonphysiological LTP induction paradigm (Albensi et al., 2007). On the other hand, TBS is considered a physiologically relevant induction paradigm because it mimics hippocampal theta rhythm thought to be necessary for memory formation (Winson, 1978; Gupta et al., 2012). However, inducing LTP at the perforant-path DGC synapse *in vitro* using TBS requires bath application of drugs that block $GABA_A$ receptors ($GABA_AR$)(Arima-Yoshida et al., 2011). Therefore, most *in vitro* studies conducting TBS LTP in the DG bath perfuse brain slices with saturating concentrations of $GABA_AR$ antagonists picrotoxin (PTX) or bicuculline which limits experimentation with the effects of inhibitory synaptic transmission on LTP (Arima-Yoshida et al., 2011). Therefore, we wanted to develop a TBS LTP induction

paradigm that produced robust LTP at perforant-path DGC synapses but also allows experimentation with the effects of inhibitory synaptic transmission on LTP and detection of pathological alterations in LTP that may be due to changes in inhibition.

Detecting the effects of GABAergic synaptic transmission on TBS-induced LTP is particularly relevant to our investigation of serotonin (5-HT) 5-HT_6 receptor antagonists and a transgenic mouse model of AD. 5-HT_6 receptor antagonists are a class of nootropic drugs thought to exert their procognitive effects primarily via a reduction to inhibitory synaptic transmission that indirectly facilities excitatory synaptic transmission (Woolley et al., 2004). In addition to testing the effects of 5-HT_6 receptor ligands in the DG physiology and function (Chapters 2 and 3 of this dissertation, respectively), we also wanted to test their effects on LTP in a transgenic animal model of AD known. J20 mice express human amyloid precursor protein (hAPP) harboring Swedish and Indiana mutations (Mucke et al., 2000) and at 3- to 5-months-of-age develop memory deficits, spontaneous seizures, and enhanced GABAergic synaptic transmission onto DGCs (Palop et al., 2007). Additionally, J20 mice also exhibit attenuated LTP at perforant path-DGC synapses compared to their (NTG littermates when tested with TBS in the presence of saturating concentration of $GABA_AR$ antagonists, precluding an understanding of the effects of GABAergic remodeling on LTP in the DG of J20 mice. Thus, our assay needed to be sensitive to increased GABA to determine whether J20 mice exhibited LTP impairments and whether 5-HT_6 receptor antagonists affected this process.

To circumvent the use of saturating concentrations of a $GABA_AR$ antagonist during TBS LTP in the DG, we established a reliable TBS stimulation paradigm at perforant-path DGC synapses in saturating concentrations of PTX in acute hippocampal

brain slices. Next, we determined the EC_{50} for PTX's effects on LTP in the DG, and hypothesized that bath application of PTX at the EC_{50} would block enough $GABA_A$Rs to facilitate TBS-induced LTP in the DG, but leave enough $GABA_A$Rs available to detect the additional effects of increased GABAergic synaptic transmission on LTP. Upon confirmation that our TBS LTP induction paradigm was sensitive to diazepam (DZP), a $GABA_A$R allosteric agonist, we subsequently tested the effects of a 5-HT_6 receptor antagonist on LTP in acute hippocampal brain slices prepared from J20 mice and their NTG littermates.

Methods

Animals

Five- to six-week-old male C57BL/6 mice (15–20 g, Charles River, Raleigh, NC, U.S.A.) were used for *in vitro* LTP. We also used 4- to 6-month-old (20–25 g) male heterozygous transgenic hAPPJ20 mice expressing human amyloid precursor protein (hAPP) with the Swedish and Indiana FAD mutations, and their nontransgenic (NTG) wild-type littermates (Jackson Laboratory, Mutant Mouse Regional Research Center) (Mucke et al., 2000). All mice were group housed in a light- and temperature-controlled (12 h on / 12 h off) environment and permitted access to food and water ad libitum throughout the study. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Utah Institutional Animal Care and Use Committee. All efforts were made to minimize the number and suffering of animals used.

Hippocampal Brain Slice Preparation

Acute hippocampal brain slices were prepared daily as described previously (West et al., 2014). Briefly, mice (20–25 g) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.), and brains were rapidly removed and submerged in ice-cold (4°C) oxygenated sucrose-based artificial cerebral spinal fluid (ACSF) bubbled with 95% O2/5% CO2. Sucrose-based ACSF contained the following (in mM): Sucrose (200.0), KCl (3.0), Na2PO4 (1.4), MgSO4 (3.0), NaHCO3 (26.0), glucose (10.0), and CaCl2 (0.5). The pH and osmolarity of the sucrose-based ACSF were adjusted to 7.30–7.35 and 290–300 mOsm, respectively, before each experiment. Next, coronal brain slices (350μm) containing dorsal hippocampus were cut using a vibrating microtome (VT1000S, Leica Microsystems Inc.). Slices were then transferred to oxygenated standard ACSF and incubated for 2 h at room temperature prior to recording. Standard ACSF was made from the same recipe as sucrose-based ACSF, only sucrose was replaced with NaCl (126.0 mM), the MgSO4 concentration lowered to 1.0 mM, and the CaCl2 concentration raised to 2.5 mM. In experiments with J20 and NTG mice, 1 mM ascorbic acid was added to the recipe for standard ACSF to prevent breakdown of 5-HT.

Long-Term Potentiation of Excitatory Synaptic Transmission

These experiments were conducted over the course of several years and the electrophysiology rig used in this study was updated midway through. The first set of experiments were conducted on a 4-channel Scientifica Slicemaster (Scientifica, Ukfield, East Sussex, U.K.), a high throughput semiautomatic brain slice recording system that conducts separate simultaneous recordings (Stopps et al., 2004). The 4-channel

Slicemaster was then upgraded to an 8-channel Scientifica Slicemaster. The major differences between the 2 recording setups, aside from simultaneously recording from 4 or 8 brain slices, are that the 4-channel Slicemaster acquired data using a pClamp 10 interfaced to a Digidata 1440A data acquisition board (Molecular Devices, Sunnyvale, CA, U.S.A.) whereas the 8-channel Slicemaster was interfaced to a Digidata 1550A data acquisition board. Additionally, the 4-channel used concentric bipolar stimulating electrodes (MCE-100; Rhodes Medical Instrument, Summerland, CA, U.S.A.), whereas the 8-channel Slicemaster used a Twisted Nichrome-Formvar stimulating electrodes. Additionally, because all stimulation strengths needed to be manually dialed-in on the 8-channel Slicemaster, adjustments of stimulation strength during TBS to strengths other than 50% on the I/O curve were substituted by increasing the pulse duration from 100 μs to 200 μs. Table A.1 indicates all TBS paradigms tested in this manuscript according to the 4- or 8-channel Slicemaster. Otherwise, all experiments were conducted as detailed below.

Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded by transferring coronal brain slices containing dorsal hippocampus from either hemisphere into the integrated brain slice chambers (IBSCs) of the Scientifica Slicemaster. All slices were perfused with regular ACSF (2 mL/min) at 30°C. In most LTP experiments, ACSF contained PTX for the duration of the entire experiments, the concentrations of which are indicated in the text and figure captions. In experiments testing the effects of 5-HT_6 receptor antagonists, ACSF contained serotonin (5-HT HCl) and the selective serotonin reuptake inhibitor (SSRI) fluoxetine HCl for the duration of the experiment to promote availability of 5-HT during studies testing the effects of 5-HT_6 receptor antagonists. The

presence and concentrations of all drugs are indicated in the text and figure captions. Stimulating electrodes were placed in the middle-third of the molecular layer in the upper blade of the DG to stimulate the medial perforant path; recording electrodes were placed in the middle-third of the molecular layer approximately 400–600 μm away from the stimulating electrode. Input/output (I/O) relationships were measured by incrementally stimulating for 100 μs in 0.5 V steps until a population spike was observed or 20 V maximum stimulus intensity was reached. Data were acquired using pClamp 10 interfaced to a Digidata 1440A or 1550A data acquisition board (4-channel and 8-channel, respectively) (Molecular Devices, Sunnyvale, CA, U.S.A.) at a sampling rate of 10 kHz, low-pass filtered at 1 kHz, and high-pass filtered at 3 Hz. The magnitude of the fEPSP was determined by measuring the 20–80% slope of the rising phase.

After obtaining fEPSPs and conducting I/O curves, baseline stimulation strength was set to 50% and slices received 1 stimulation pulse every 30 s for 30 min. Drugs were applied via bath exchange for 20 min prior to the LTP induction stimulus. LTP was induced via TBS or HFS. The LTP induction parameters varied and are indicated in Table A.1 as well as the figure captions using the following nomenclature: number of pulses X number of trains X number of bursts. Pulse duration was either 100 or 200 μs; intra-train frequency was always 100 Hz; intertrain frequency was 5 Hz for all TBS stimulations paradigms; interburst interval for TBS was always 20 s, and 10 or 60 s for HFS. All fEPSPs were normalized to the average slope of the last 4 fEPSPs prior to TBS and are represented as the mean ± standard error of the mean (SEM). fEPSP amplitudes that exhibited drift during the 30 min baseline > ± 20 % were excluded.

Chemicals and Drugs

Unless indicated otherwise, all chemicals and drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of SB-399885 (Tocris Bioscience, Minneapolis, MN, U.S.A.) DZP, 5-HT, fluoxetine were dissolved in ddH_2O. PTX was dissolved in DMSO. All drugs were made into stock solutions ≥ 1000-fold their final concentrations and stored at -20°C. On the day of the experiment, individual aliquots were defrosted and dissolved in ACSF to the concentration indicated in each figure.

Statistics

Statistical comparisons were made by comparing the mean normalized slope of each condition over the last 30 min of recording using a two-way analysis of variance (ANOVA) with repeated measures. When drawing comparisons across 4 groups, ACSF- and SB-399885-treated slices from J20 and NTG mice, a two-way ANOVA with Bonferroni's multiple comparisons was used to compare that mean normalized slope of the last 5 fEPSPs. Post-tetanic potentiation (PTP) time constants (tau) were also measured; these were represented as best-fit values (95% confidence interval [CI]) from one-phase exponential decay regression functions. The EC_{50} for PTX's effects on LTP were determined by fitting a nonlinear regression to the PTX concentration-response LTP, composed of the mean normalized slope of the last 5 fEPSPs for each PTX concentration tested. All N values are presented as the number of slices tested / from the number of animals slices were prepared from. *, **, or *** indicate a *p*-value of < 0.05, < 0.01, and < 0.001, respectively.

Results

4x2x5 at 70% in 50 μM PTX Is a Reliable TBS LTP Induction Paradigm

First, we wanted to determine a reliable TBS LTP induction stimulus at perforant path-DGC synapses in the presence of 50 μM PTX. We attempted a variety of stimulation parameters that varied by train and burst number, all of which are detailed in Table A.1. One of the first TBS paradigms that produced reliable LTP was 4x2x5 adjusted to 70% of the stimulation strength needed to produce a population spike, as determined from I/O relationships. We also tested the dependency of our paradigm on adjusting the stimulation strength by comparing slices stimulated at 70% (n = 10 / 2) it with LTP produced from 4x2x5 at 50% (n = 6 / 1) (Figure A.1). Two-way repeated measures ANOVA comparing the last 30 min revealed a significant effect of stimulation strength ($F_{(1, 826)} = 6.15$; $p < 0.05$) and interaction between stimulation strength and time ($F_{(59, 826)} = 1.85$; $p < 0.001$). Stimulation strength also significantly affected the PTP decay constant (Stimulation Strength: 70% tau: 23.0 (95% CI 14.6–54.5); 50% tau: 7.2 (95% CI 4.8–14.3), $p < 0.05$, nonlinear regression). These results suggest that our stimulation paradigm, 4x2x5, produces robust LTP when the stimulation strength is set to 70% on the I/O curve. All experiments conducted on the 4-channel Slicemaster hereinafter used the TBS LTP induction paradigm 4x2x5 at 70%.

The EC_{50} for PTX's Effects on the TBS LTP Induction Paradigm 4x2x5 at 70% Is 4 μM

Next, we conducted a concentration-response for the effects of PTX on LTP using our TBS-LTP induction stimulus 4x2x5 at 70%. Time course LTP and the concentration-response curve for the effects of 0, 1, 3, 10, 30, and 100 μM PTX on TBS-LTP are plotted in Figure A.2. Nonlinear regression of PTX's concentration-response on LTP revealed an EC_{50} of 3.8 μM PTX. Future studies rounded the EC_{50} for PTX up to 4 μM.

The TBS LTP Induction Paradigm 4x2x5 at 70% in 4 μM PTX Is Sensitive to DZP

Next, we tested whether the TBS LTP induction paradigm 4x2x5 at 70% in the presence of 4 μM PTX was sensitive to increased GABAergic synaptic transmission by bath perfusing 50 μM DZP for 20 min before prior to TBS (Figure A.3). Two-way ANOVA with repeated measures revealed DZP-treated slices (n = 12 / 3) exhibited significantly attenuated LTP compared to ACSF-treated slices (n = 10 / 3) with significant effects for drug, time, and interaction (Drug: $F_{(1, 1200)} = 4.75$, $p < 0.05$; Time: $F_{(60, 1200)} = 12.70$; $p < 0.001$; Interaction: $F_{(60, 1200)} = 2.76$; $p < 0.001$). Moreover, nonlinear regression showed that DZP significantly reduced the PTP decay constant (Control tau: 22.6 (95% CI 9.9–15.8) and DZP tau: 12.1 (95% CI 16.2–37.3), $p < 0.05$). Together, these results suggest that the TBS LTP induction paradigm 4x2x5 at 70% stimulation strength in the presence of 4 μM PTX is sensitive to pharmacologically increased GABAergic synaptic transmission.

LTP in J20 and NTG Mice Did Not Differ and Was Unaffected by SB-399885

Once we established a TBS LTP induction paradigm sensitive to pharmacologically increased inhibitory synaptic transmission, we tested the effects of the 5-HT_6 receptor antagonist SB-399885 (1 μM) on LTP at perforant-path DGC synapses in acute hippocampal brain slices prepared from a transgenic animal model of AD, J20 mice, and their NTG littermates (Figure A.4). To increase extracellular concentrations of 5-HT, these experiments were conducted in the presence of 4 μM PTX, as well as 1 μM 5-HT and 10 μM fluoxetine. Two-way ANOVA with repeated measures revealed no significant effects in J20 or NTG mice (Figure A.4A) Additionally, two-way ANOVA comparing the mean normalized slope of the last 5 fEPSPs across the 4 treatment groups revealed no significant effects. Thus, J20 and NTG mice did not exhibit significant differences in LTP, and SB-399885 had no effect on LTP in either group.

4x2x5 at 70% Is No Longer Sensitive to DZP on the 8-Channel Slicemaster

At this time, the 4-channel Scientifica Slicemaster was updated to the 8-channel Slicemaster and we wanted to test whether the TBS LTP induction paradigm 4x2x5 at 200 μs stimulation duration produced LTP that is sensitive to pharmacologically increased $GABA_AR$ receptor-mediated inhibition (Figure A.5). Two-way repeated measures ANOVA revealed that LTP on the 8-channel Slicemaster was no longer sensitive to the effects of 50 μM DZP when tested in the presence of 1, 4, or 10 μM PTX (Figure A.5A–C). We did observe a significant decrease in the PTP decay constant in

DZP-treated slices at 10 μM PTX (Figure A.5C; Control tau: 15.5 (95% CI 12.4–20.5) and DZP tau: 44.0 (95% CI 16.9–∞), $p < 0.05$, nonlinear regression).

Since our TBS LTP paradigm was no longer sensitive to pharmacologically increased $GABA_AR$-mediated inhibitory synaptic transmission, the effects of DZP on LTP were tested in a variety of TBS and HFS stimulation paradigms; see Table A.1 for details. Two-way repeated measures ANOVA revealed none of these paradigms produced LTP that was significantly attenuated by DZP. Thus, we were unable to establish a TBS or HFS LTP stimulation paradigm on the 8-channel Slicemaster that was sensitive to pharmacologically increased GABAergic synaptic transmission.

LTP in J20 and NTG Mice Did Not Differ When Tested in the Presence of 4 or 50 μM PTX

Despite the inability to implement an LTP stimulation paradigm on the updated 8-channel Slicemaster that was sensitive to increased GABAergic synaptic transmission, we tested the TBS LTP paradigm 4x2x5 at 200 μs LTP in the presence of 4 and 50 μM PTX in slices from J20 and NTG mice (Figure A.6). Two-way ANOVA with repeated measures revealed no significant effects at either 4 or 50 μM PTX. PTP comparison using nonlinear regression revealed no significant differences in the decay constants, and an unpaired Student's *t* test comparing the mean normalized slope in the first 2 min following TBS showed no PTP differences between NTG and J20 mice in 4 or 50 μM PTX. These results suggest that J20 mice do not have significant LTP deficits at perforant-path DGC synapses in the absence of GABAergic synaptic transmission, or when GABAergic synaptic transmission is partially blocked.

Discussion

This study tested a variety of TBS and HFS LTP induction paradigms at perforant path-DGC synapses in acute hippocampal brain slices on 2 separate electrophysiology rigs. On the 4-channel Slicemaster, the TBS LTP induction paradigm 4x2x5 at 70% stimulation strength produced reliable LTP at perforant path-DGC synapses in the presence of 50 μM PTX. Next, we determined that the EC_{50} for PTX's effect on LTP using our TBS paradigm was 4 μM, and that these conditions were sensitive to increased $GABA_AR$ receptor-mediated inhibition via 50 μM DZP. Experiments in slices from J20 and NTG mice suggested they do not have differences in LTP in the presence of 4 μM PTX, and the 5-HT_6 receptor antagonist SB-399885 had no effect on LTP in either genotype. On our 8-channel Slicemaster, none of the TBS or HFS LTP induction paradigms tested were attenuated by DZP. We did, however, retest the effects of our initial TBS LTP induction paradigm 4x2x5 at 200 μs stimulation duration in slices from J20 and NTG mice. Again, we observed no significant differences in LTP between J20 and NTG mice in either 4 or 50 μM PTX. Together, these results suggest it is possible to reliably produce TBS-induced LTP at perforant-path DGC synapses that is sensitive to increased $GABA_AR$-mediated inhibitory synaptic transmission. Furthermore, this assay may be useful for determining the effects of drugs or disease states that affect LTP via GABAergic mechanisms.

Rationale for these experiments is rooted in the idea that changes in LTP associated with a drug or disease state may be predictive of their effects on learning and memory. For instance, a number of drugs or genetic manipulations that improve memory also increase or restore pathologically attenuated LTP (Lee and Silva, 2009; Lynch and

Gall, 2013; Lynch et al., 2014). Moreover, ASDs such as carbamazepine, phenobarbital, phenytoin, and valproate have all been shown to impair memory, as well as attenuate hippocampal LTP *in vitro* (Meador et al., 1993; Meador et al., 1995; Lee et al., 2003; Meador et al., 2005; Salinsky et al., 2005; West et al., 2014). Interestingly, the anticonvulsant LEV, which has a more benign cognitive profile, did not impair hippocampal LTP (West et al., 2014). Lastly, many neurological disorders that present with memory deficits also exhibit abnormalities in LTP, in tissue from both patients and animals models of disease (Beck et al., 2000; Palop et al., 2007; Kleschevnikov et al., 2012). Although not without exception, these studies support the idea that drug- or disease state-induced alterations in LTP can be predictive of their beneficial or deleterious effects on learning and memory.

The present study developed a screening assay to test the effects of drugs and disease states that affect LTP at perforant-path DGC synapses via GABAergic mechanisms, which was successful in the presence of DZP. Inhibitory synaptic transmission plays a critical role in the fine-tuning of synaptic transmission required for synaptic plasticity and memory formation (Letzkus et al., 2015), and drugs and disease states, as well as genetic and optogenetic manipulations that impact GABAergic synaptic transmission can also affect learning and memory (Palop et al., 2007; Cui et al., 2008; Braudeau et al., 2011; Andrews-Zwilling et al., 2012; Kleschevnikov et al., 2012; Verret et al., 2012). Thus, developing a physiologically relevant TBS LTP paradigm sensitive to changes in inhibition may be useful in the discovery of nootropic drugs that improve learning and memory via their effects on inhibitory synaptic transmission, as well as drugs that may adversely impact cognition. However, detecting the effects of inhibitory

synaptic transmission on LTP *in vitro*, and thus its use in drug development, is hampered in the DG when using a physiologically relevant LTP induction paradigm such as TBS. Our study circumvented this limitation by testing our TBS paradigm at PTX's EC_{50} and revealing its sensitivity to increased GABAergic synaptic transmission with the positive-allosteric $GABA_AR$ modulator DZP.

Despite the use of a TBS LTP induction paradigm sensitive to DZP, we did not detect any differences in LTP between J20 mice and their NTG littermates when GABAergic synaptic transmission is presumably only partially blocked by 4 μM PTX. Interestingly, these results conflict with results reported by Palop et al. (2007), illustrating that J20 mice exhibit attenuated TBS-induced LTP at perforant path-DGC synapses when GABAergic synaptic transmission was completely blocked. We hypothesized that slices from J20 mice would exhibit attenuated LTP in the presence of 4 μM PTX since J20 mice exhibit increased inhibitory synaptic transmission onto DGCs (Palop et al., 2007), and increases in inhibitory synaptic transmission are thought to attenuate LTP. One interpretation of our results is that enhanced GABAergic synaptic transmission in J20 mice may correct LTP deficits reported in the absence of inhibitory synaptic transmission. Although our study did not detect a significant difference in LTP in J20 mice when inhibitory synaptic transmission was completely blocked (Figure A.6B), we may have been able to replicate the finding by Palop et al. (2007) had we tested a greater number of slices. However, our experiments were limited by the availability of J20 mice. Together, these results, combined with Palop et al's. (2009) findings, suggest that J20 mice have LTP deficits at perforant-path DGC synapses independent of GABAergic synaptic transmission. Thus, testing LTP in J20 mice with a paradigm sensitive to

increased GABAergic synaptic transmission may have been useful in obtaining a better understanding of the mechanisms underlying LTP deficits in J20 mice.

In addition to testing our TBS LTP induction paradigm in a mouse model of AD, we also tested the effects of the 5-HT_6 receptor antagonist SB-399885 on LTP in brain slices prepared from J20 mice and their NTG littermates. We hypothesized that SB-399885 would increase LTP in the DG because of its widely reported nootropic effects in naïve, aged, and amnestic rodents in a variety of cognitive tests (Fone, 2008). Additionally, drugs that attenuate inhibitory synaptic transmission typically increase LTP (Arima-Yoshida et al., 2011), and results from Chapter 2 of this dissertation suggest that 5-HT_6 receptor antagonists dampen inhibitory synaptic transmission in the DG. Lastly, West et al. (2009) reported that 5-HT_6 receptor activation attenuated LTP at CA3-CA1 synapses. However, our results showed that SB-399885 had no effect on LTP in slices from NTG or J20 mice. One possible explanation for these results could be that 5-HT_6 receptor antagonist effects on LTP in the DG are subtle, and our test was not sensitive enough to detect 5-HT_6 receptor-mediated changes to LTP in the DG. Another possibility is that the EC_{50} for PTX's effects in NTG and J20 mice may be left-shifted compared to the EC_{50} in naïve C57BL/6 mice (which the PTX concentration-response was initially conducted in) such that 4 μM PTX blocked more than 50% of $GABA_A$Rs in the DG, leaving less $GABA_A$Rs available to mediate the effects of SB-399885. Although additional studies are needed, it is also possible that SB-3998855 does not affect LTP in the DG. Future experiments could test whether SB-399885 affects LTP in the DG in perhaps lower concentrations of PTX, or using TBS with different stimulation parameters.

In regards to the validity of LTP as an assay used to predict performance on learning and memory tasks, it is encouraging that our results were trending on detecting an attenuation in LTP in the DG of J20 mice when tested in the presence of 50 μM PTX, as these results would replicate findings published by Palop et al. (2009). However, it is discouraging that we did not observe an effect of SB-399885 on LTP in J20 or NTG mice. Results in Chapter 3 of this dissertation show that SB-399885 significantly improved the performance of NTG during the metric task, a spatial pattern processing paradigm thought to rely on DG function; and yet, we did not detect any effects of SB-399885 on LTP. Although other factors, including SB-399885's potential sedative effects, may have influenced the performance of NTG mice during the metric task, the memory enhancing effects of SB-399885 and other 5-HT_6 receptor antagonists are well-established. Thus, results in the present study were not predictive of the cognitive enhancing effects of SB-399885, at least in the DG, and suggest that the conditions of our assay may need to be modified to better predict the procognitive effects of drugs.

Another caveat to the present study is that sensitivity of our LTP induction paradigm to DZP was no longer detected on the 8-channel Slicemaster. This could have been due to a number of reasons pertaining to the stimulator and stimulating electrodes that changed when the 4-channel Slicemaster was updated to the 8-channel Slicemaster. However, it is unlikely that the 8-channel Slicemaster is incapable of producing LTP that is sensitive to changes in inhibition. Future studies should test a variety of TBS LTP induction paradigms and test their sensitivity to changes in GABAergic synaptic transmission using DZP, and perhaps additional compounds that affect GABAergic synaptic transmission, such as the ASDs gabapentin or tiagabine. Moreover, an important

control experiment in the present study would be to test the effects of DZP or other GABA-enhancing drugs on LTP in saturating concentrations of PTX to rule out nonspecific effects. Additionally, the number of slices and animals tested in the some of these paradigms was limited. Future studies should test each TBS paradigm more comprehensively using at least 12 slices from 4 animals per condition.

In conclusion, results in this study suggest it is possible to develop a TBS paradigm that produces reliable LTP at perforant path-DGC synapses sensitive to pharmacologically increased inhibitory synaptic transmission. Although we were unable to reproduce these results on the 8-channel Slicemaster, and we did not observe any LTP differences in slices prepared from J20 and NTG mice, as well as 5-HT_6 receptor antagonist-treated slices, we did validate our TBS LTP paradigm, showing sensitivity to the positive allosteric $GABA_AR$ agonist DZP. Given the predictive validity of LTP and its effects on memory, and the critical role inhibitory synaptic transmission plays in memory formation, alterations in LTP resulting from a drug or disease state that exerts its effects via inhibitory synaptic transmission may be a predictive biomarker for their enhancing or deleterious effects on learning and memory. Coupled with an 8-channel recording system such as the Slicemaster, *in vitro* LTP is suitable to high-throughput drug screening that may be a valuable tool in the discovery of novel therapeutics for the treatment cognitive dysfunction in disease.

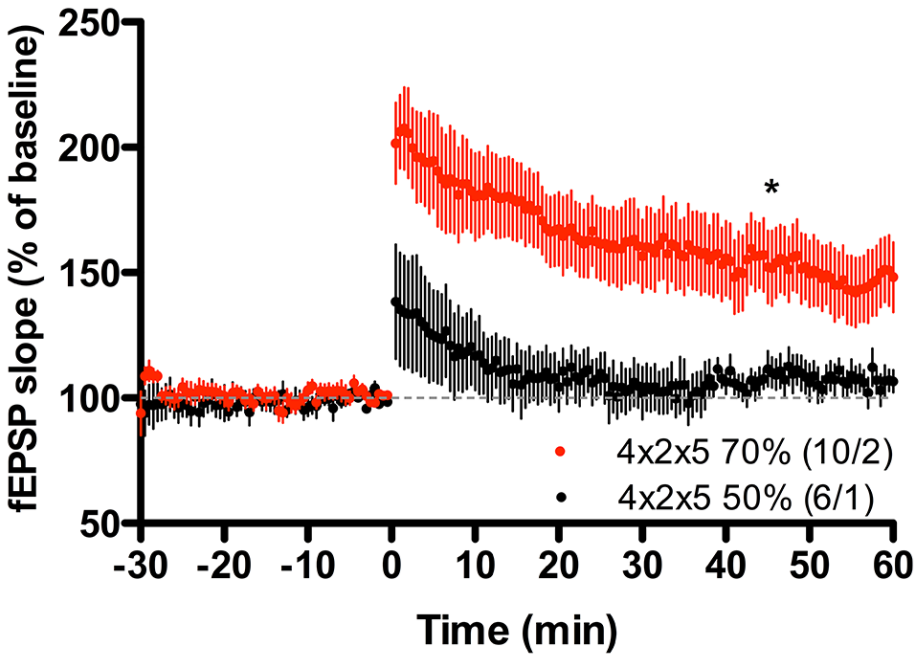


Figure A.1. The TBS-LTP induction paradigm 4x2x5 requires the stimulation strength to be set at 70% on the I/O curve to achieve LTP. fEPSP slope as a function of time. fEPSP slopes are normalized to the average of the last 4 fEPSP slopes before TBS. Brain slices were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 with the stimulation strength set to either 70% (red, n = 10 slices from 2 animals) or 50% (black, n = 6 slices from 1 animal) in the presence of 50 µM PTX. TBS at 70% stimulation strength produced LTP that was significantly greater slices that received TBS at 50% stimulation strength (* $p < 0.05$, two-way ANOVA with repeated measures of last 30 min).

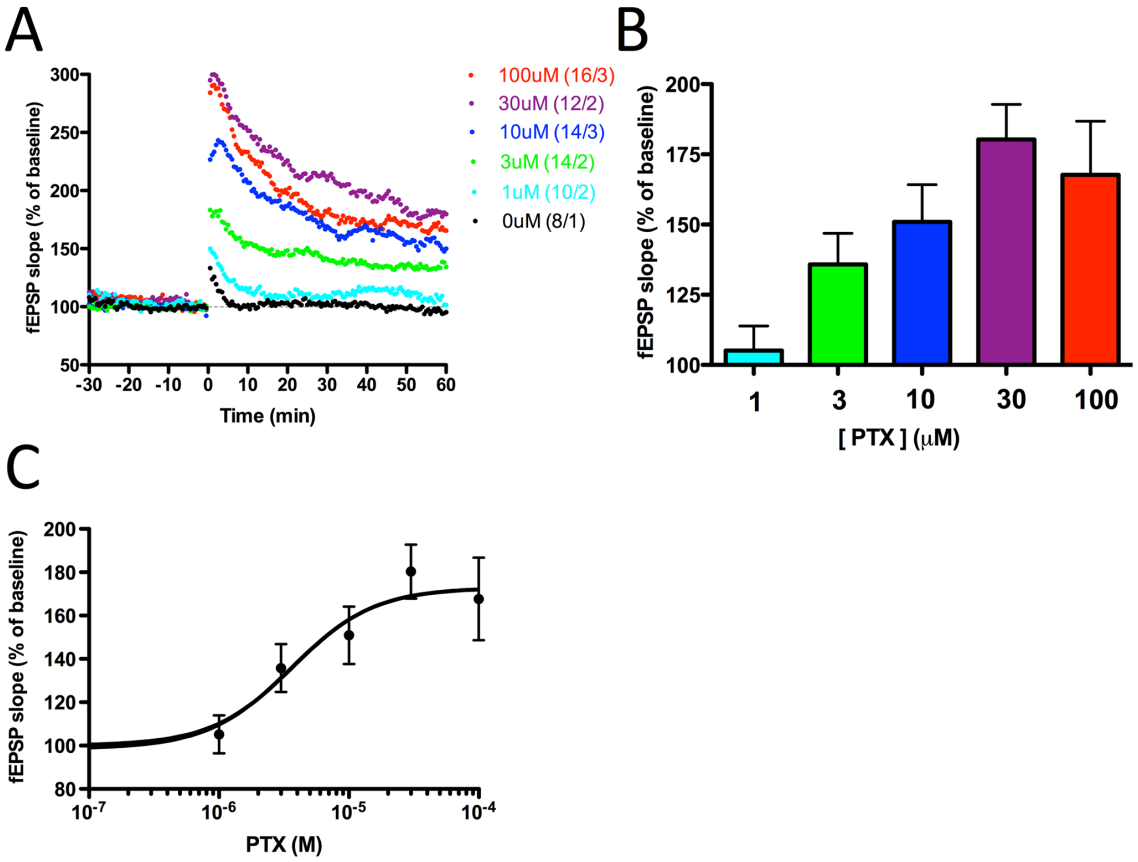


Figure A.2. PTX concentration-response reveals an EC_{50} of 4 μM for its effects on LTP in the DG. A) fEPSP slope as a function of time. Brain slices in the presence of either 0, 1, 3, 10, 30, or 100 μM PTX were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 at either 70% (*n* = number of slices / number of animals). fEPSP slopes are normalized to the average of the last 4 fEPSPs before TBS. B) the average normalized slope of the last 5 fEPSPs for each concentration of PTX presented as a bar graph. C) Concentration-response curve for the last 5 fEPSPs. Nonlinear regression revealed an EC_{50} of 4 μM for PTX effects on LTP at perforant path-DGC synapses.

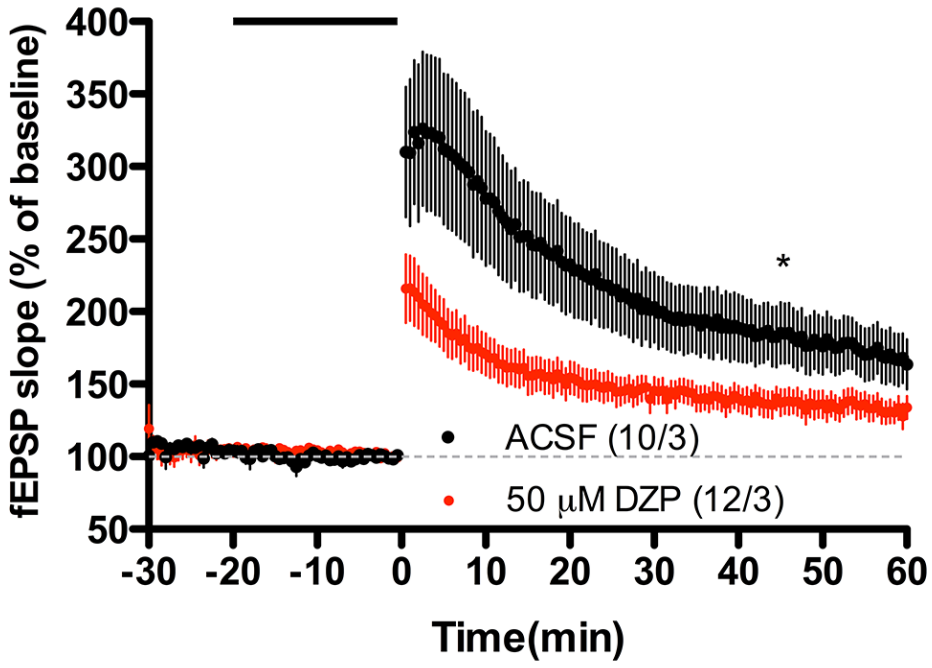


Figure A.3. LTP induced using the TBS paradigm 4x2x5 at 70% in 4 μM PTX is attenuated by DZP. fEPSP slopes are normalized to the average of the last 4 fEPSPs before TBS. Brain slices in the presence of 4 μM PTX were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 at either 70% were exposed to 50 μM DZP (red, n = 12 slices from 3 animals) or regular ACSF (black, n = 10 slices from 3 animals) for 20 min before TBS (blue bar). Slices exposed to 50 μM DZP exhibited significantly attenuated LTP compared to slices exposed to regular ACSF (* $p < 0.05$, two-way repeated measures ANOVA of last 30 min). Additionally, DZP significantly reduced the PTP decay constant (Control tau: 22.6 (95% CI 16.2–37.3) and DZP tau: 12.1 (95% CI 9.9–15.8), $p < 0.05$, nonlinear regression).

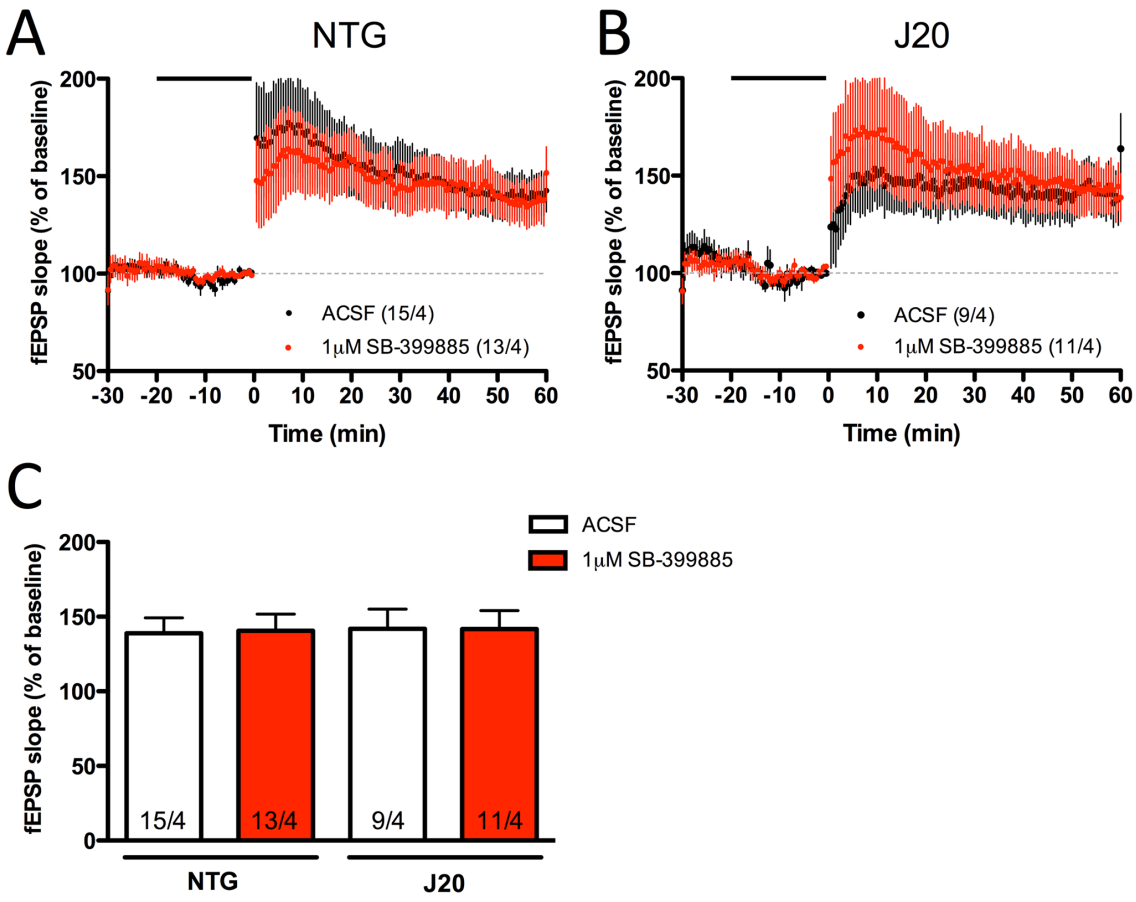


Figure A.4. J20 and NTG mice exhibit similar LTP in the DG that is unaffected by SB-399885. A) fEPSP slope as a function of time. Brain slices from NTG mice were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 at 70% in the presence of 4 μM PTX, 1 μM 5-HT, and 10 μM fluoxetine. For 20 min prior to TBS (black bar), slices were exposed to 1 μM SB-399885 (red, n = 13 slices from 4 animals) or regular ACSF (black, n = 15 slices from 4 animals). fEPSP slopes are normalized to the average of the last 4 fEPSPs before TBS. Compared with ACSF-treated slices, 1 μM SB-399885 had no effect on LTP in NTG mice ($p > 0.05$, two-way repeated measures ANOVA of last 30 min). B) Brain slices from J20 mice were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 at 70% in the presence of 4 μM PTX, 1 μM 5-HT, and 10 μM fluoxetine. For 20 min before TBS (black bar), slices were exposed to 1 μM SB-399885 (red, n = 11 slices from 4 animals) or regular ACSF (black, n = 9 slices from 4 animals). Compared with ACSF-treated slices, 1 μM SB-399885 had no effect on LTP in J20 mice ($p > 0.05$, two-way repeated measures ANOVA of last 30 min). C) Bar graph illustrating the means of the normalized fEPSP slope by treatment group. No significant effects were detected ($p > 0.05$, two-way ANOVA).

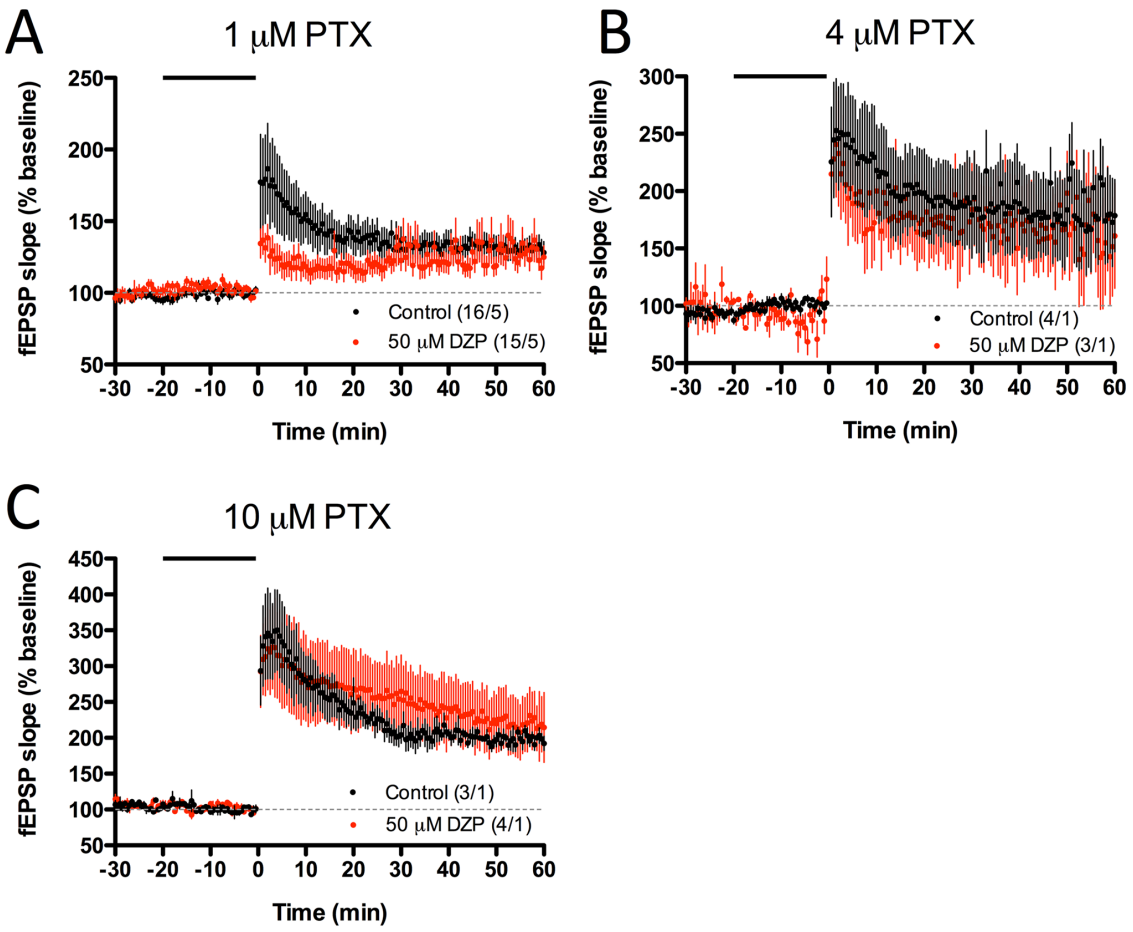


Figure A.5. The TBS-LTP induction stimulus 4x2x5 with a 200 μs pulse duration was unaffected by DZP on the upgraded 8-channel Slicemaster. A) fEPSP slope as a function of time. Brain slices prepared from C57BL/6 mice in the presence of 1 μM were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 with a 200 μs pulse duration. For 20 min before TBS (blue bar), slices were exposed to 50 μM DZP (red, n = 15 slices from 5 animals) or regular ACSF (black, n = 16 slices from 5 animals). Slices exposed to 50 μM DZP did not exhibit significantly different LTP compared to ACSF-treated slices ($p > 0.05$, two-way repeated measures ANOVA of last 30 min). B) In the presence of 4 μM PTX, slices exposed to 50 μM DZP (red, n = 3 slices from 1 animal) did not exhibit significantly different LTP compared to slices exposed to ACSF (black, n = 4 slices from 1 animal) ($p > 0.05$, two-way repeated measures ANOVA of last 30 min). C) In the presence of 10 μM PTX, slices exposed to 50 μM DZP (red, n = 3 slices from 1 animal) did not exhibit significantly different LTP compared to ACSF-treated slices (black, n = 4 slices from 1 animal) ($p > 0.05$, two-way repeated measures ANOVA of last 30 min). Additionally, in 10 μM PTXDZP significantly reduced the PTP decay constant (Control tau: 15.5 (95% CI 12.4–20.5); DZP tau: 44.0 (95% CI 16.9–infinity), $p < 0.05$, nonlinear regression).

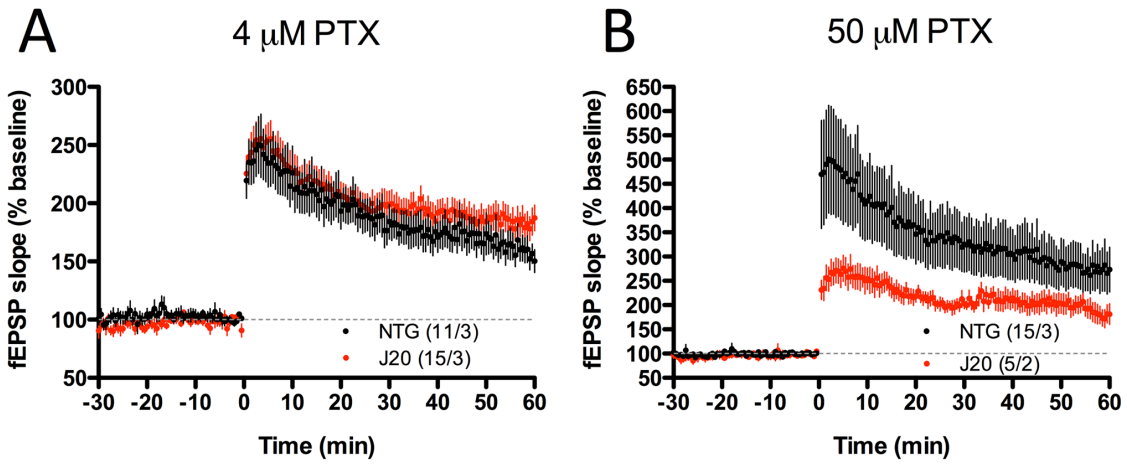


Figure A.6. J20 and NTG mice exhibit similar LTP in the DG under partial and complete blockade of $GABA_A$ receptors. A) fEPSP slope as a function of time. Brain slices from NTG (black, n = 11 slices from 3 animals) and J20 mice (red, n = 15 slices from 3 animals) were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 with a 200 μs pulse duration in the presence of 4 μM PTX. fEPSP slopes are normalized to the average of the last 4 fEPSPs before TBS. No group difference in LTP were detected (p > 0.05, two-way repeated measures ANOVA of last 30 min). B) Brain slices from NTG (black, n = 11 slices from 3 animals) and J20 mice (red, n = 5 slices from 2 animals) were stimulated at 0 min using the TBS-LTP induction paradigm 4x2x5 with a 200 μs pulse duration in the presence of 50 μM PTX. No difference in LTP was detected (p > 0.05, two-way repeated measures ANOVA of last 30 min).

Table A.1

Summary of TBS and HFS LTP induction paradigms tested in the DG.

All experiments were conducted in acute hippocampal brain slices prepared from C57BL/6 mice. Abbreviations: 4 Ch = 4-channel Slicemaster; 8 Ch = 8-channel Slicemaster; C = control (always standard ACSF); D = drug (always 50 μM Diazepam); HFS = high-frequency stimulation; I/O = input-output curve; ISI = interstimulus interval; LTD = long-term depression; LTP = long-term potentiation; N = number of slices in control condition, number of slices in drug condition / from the number of animals tested; PTD = post-tetanic depression; PTP = post-tetanic potentiation; PTX = picrotoxin; TBS = theta-burst stimulation.

Slice-master	LTP Induction Stimulus: Pulses x Trains x Bursts		Pulse Duration (μs)	Strength On I/O Curve (%)	ISI (s)	PTX [μM)	N	Results
4 Ch	TBS	4x4x2	100	50	20	50	4 / 1	Flat
		4x4x3	100	50	20	50	4 / 1	
		4x4x4	100	50	20	50	4 / 1	
		4x10x10	100	50	20	50	2 / 1	PTD
		4x10x2	100	50	20	50	2 / 1	
		4x2x2	100	50	20	50	4 / 1	PTP, flat
		4x2x5	100	70	20	50	10 / 2	LTP
		4x2x2	100	70	20	50	10, 12 / 3	C: LTP > D: LTP
		4x2x5	100	50	20	50	6 / 1	PTP, flat
8 Ch	TBS	4x2x2	200	50	20	1	16, 15 / 5	C and D LTP; No significant differences
		4x2x5	200	50	20	4	4, 3 / 1	
		4x2x2	200	50	20	10	3, 4 / 1	
		4x4x4	100	50	20	4	17, 12 / 5	
		4x4x4	100	50	20	10	5, 5 / 2	
		4x4x4	100	50	20	1	7, 5 / 2	Flat
	HFS	50x1x4	100	50	10	0	3, 2 / 1	Flat
		50x1x4	200	50	10	0	3, 3 / 1	C: LTD D: Flat
		50x1x4	200	50	60	0	6 / 1	PTP no LTP
		50x1x2	200	50	60	0.3	6 / 1	
		50x1x2	200	50	60	0.3	2, 3 / 1	

References

Albensi BC, Oliver DR, Toupin J, Odero G (2007) Electrical stimulation protocols for hippocampal synaptic plasticity and neuronal hyper-excitability: are they effective or relevant? Exp Neurol 204:1-13.

Andrews-Zwilling Y, Gillespie AK, Kravitz AV, Nelson AB, Devidze N, Lo I, Yoon SY, Bien-Ly N, Ring K, Zwilling D, Potter GB, Rubenstein JL, Kreitzer AC, Huang Y (2012) Hilar GABAergic interneuron activity controls spatial learning and memory retrieval. PLoS One 7:e40555.

Arima-Yoshida F, Watabe AM, Manabe T (2011) The mechanisms of the strong inhibitory modulation of long-term potentiation in the rat dentate gyrus. Eur J Neurosci 33:1637-1646.

Beck H, Goussakov IV, Lie A, Helmstaedter C, Elger CE (2000) Synaptic plasticity in the human dentate gyrus. J Neurosci 20:7080-7086.

Braudeau J, Delatour B, Duchon A, Pereira PL, Dauphinot L, de Chaumont F, Olivo-Marin JC, Dodd RH, Herault Y, Potier MC (2011) Specific targeting of the GABA-A receptor alpha5 subtype by a selective inverse agonist restores cognitive deficits in Down syndrome mice. J Psychopharmacol 25:1030-1042.

Chapman CA, Perez Y, Lacaille JC (1998) Effects of GABA(A) inhibition on the expression of long-term potentiation in CA1 pyramidal cells are dependent on tetanization parameters. Hippocampus 8:289-298.

Cui Y, Costa RM, Murphy GG, Elgersma Y, Zhu Y, Gutmann DH, Parada LF, Mody I, Silva AJ (2008) Neurofibromin regulation of ERK signaling modulates GABA release and learning. Cell 135:549-560.

Fone KC (2008) An update on the role of the 5-hydroxytryptamine6 receptor in cognitive function. Neuropharmacology 55:1015-1022.

Gupta AS, van der Meer MA, Touretzky DS, Redish AD (2012) Segmentation of spatial experience by hippocampal theta sequences. Nat Neurosci 15:1032-1039.

Kleschevnikov AM, Belichenko PV, Faizi M, Jacobs LF, Htun K, Shamloo M, Mobley WC (2012) Deficits in cognition and synaptic plasticity in a mouse model of Down syndrome ameliorated by GABAB receptor antagonists. J Neurosci 32:9217-9227.

Lee S, Sziklas V, Andermann F, Farnham S, Risse G, Gustafson M, Gates J, Penovich P, Al-Asmi A, Dubeau F, Jones-Gotman M (2003) The effects of adjunctive topiramate on cognitive function in patients with epilepsy. Epilepsia 44:339-347.

Lee YS, Silva AJ (2009) The molecular and cellular biology of enhanced cognition. Nat Rev Neurosci 10:126-140.

Letzkus JJ, Wolff SB, Luthi A (2015) Disinhibition, a circuit mechanism for associative learning and memory. Neuron 88:264-276.

Lynch G, Cox CD, Gall CM (2014) Pharmacological enhancement of memory or cognition in normal subjects. Front Syst Neurosci 8:90.

Lynch G, Gall CM (2013) Mechanism based approaches for rescuing and enhancing cognition. Front Neurosci 7:143.

Meador KJ, Loring DW, Abney OL, Allen ME, Moore EE, Zamrini EY, King DW (1993) Effects of carbamazepine and phenytoin on EEG and memory in healthy adults. Epilepsia 34:153-157.

Meador KJ, Loring DW, Moore EE, Thompson WO, Nichols ME, Oberzan RE, Durkin MW, Gallagher BB, King DW (1995) Comparative cognitive effects of phenobarbital, phenytoin, and valproate in healthy adults. Neurology 45:1494-1499.

Meador KJ, Loring DW, Vahle VJ, Ray PG, Werz MA, Fessler AJ, Ogrocki P, Schoenberg MR, Miller JM, Kustra RP (2005) Cognitive and behavioral effects of lamotrigine and topiramate in healthy volunteers. Neurology 64:2108-2114.

Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G, Hu K, Kholodenko D, Johnson-Wood K, McConlogue L (2000) High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci 20:4050-4058.

Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Bien-Ly N, Yoo J, Ho KO, Yu GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L (2007) Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. Neuron 55:697-711.

Salinsky MC, Storzbach D, Spencer DC, Oken BS, Landry T, Dodrill CB (2005) Effects of topiramate and gabapentin on cognitive abilities in healthy volunteers. Neurology 64:792-798.

Stopps M, Allen N, Barrett R, Choudhury HI, Jarolimek W, Johnson M, Kuenzi FM, Maubach KA, Nagano N, Seabrook GR (2004) Design and application of a novel brain slice system that permits independent electrophysiological recordings from multiple slices. J Neurosci Methods 132:137-148.

Verret L, Mann EO, Hang GB, Barth AM, Cobos I, Ho K, Devidze N, Masliah E, Kreitzer AC, Mody I, Mucke L, Palop JJ (2012) Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. Cell 149:708-721.

West PJ, Saunders GW, Remigio GJ, Wilcox KS, White HS (2014) Antiseizure drugs differentially modulate theta-burst induced long-term potentiation in C57BL/6 mice. Epilepsia 55:214-223.

Winson J (1978) Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science 201:160-163.

Woolley ML, Marsden CA, Fone KC (2004) 5-ht6 receptors. Curr Drug Targets CNS Neurol Disord 3:59-79.